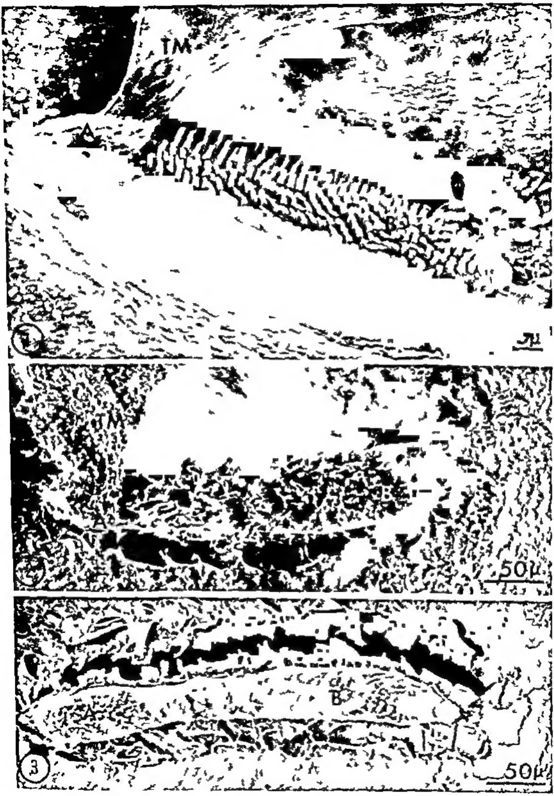
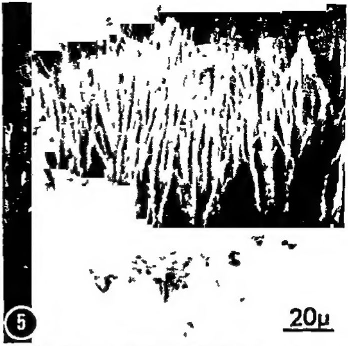


Fig 1 Normal bas lar pap lla with ntact tectorial membrane (*TM*) and ventral type A sensory cells (*A*) and dorsal type B cells (*B*) ×300

Fig 2 Moderately damaged bas lar pap lla from a l zard treated for 14 days Intact tectorial membrane (*TM*) and type A (*A*) and type B (*B*) sensory cells ×300

Fig 3 Severely damaged basilar pap lla from an an mal treated for 21 days The ventral type A cell population (*A*) s relat vely intact wh le only occasional type B cells remain (*B*) ×300



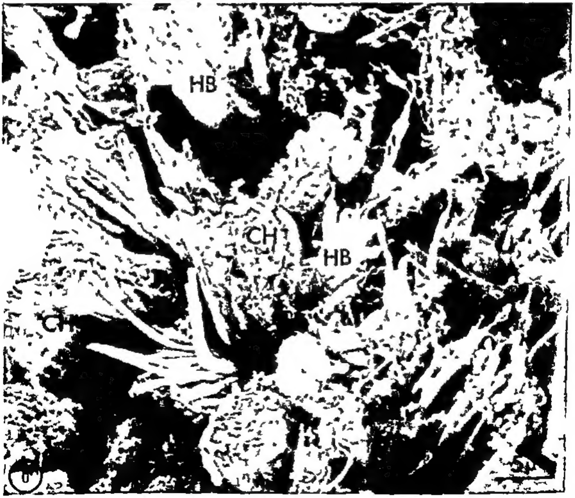


Fig 4 Normal type A sensory hair bundles ×8000
Fig 5 Normal type B sensory hair bundles ×550
Fig 6 Moderately damaged basilar papilla with fused hair bundles (*HB*) and cytoplasmic herniations (*CH*) in the dorsal type B sensory cell area ×3400

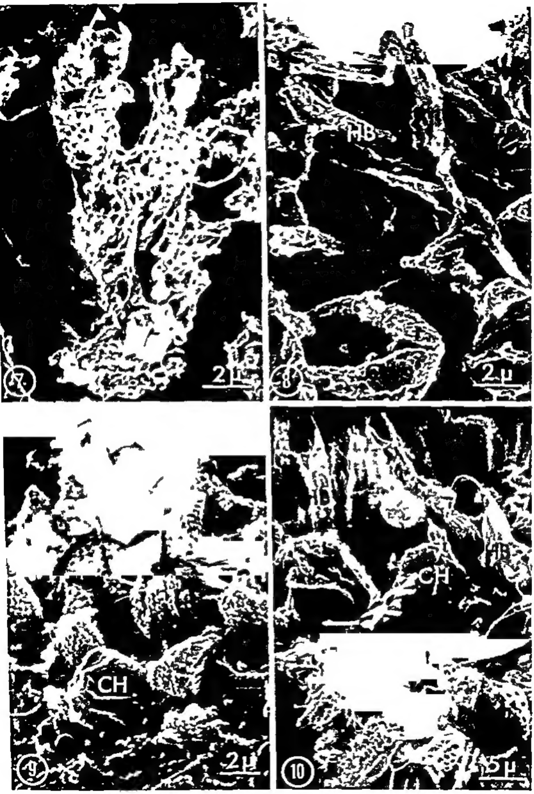


Fig 7 Fused giant hair of a type B sensory cell ×6 100
Fig 8 Distorted hair bundles (*HB*) and cytoplasmic herniations in a moderately damaged basilar papilla ×5 900
Fig 9 Moderately damaged type A sensory cells with cytoplasmic herniations (*CH*) and collapsed hair bun dles ×4 800
Fig 10 Cytoplasmic herniations (*CH*) and fused h bundles (*HB*) in a moderately damaged organ the free floating cytoplasm ×2 300

A cell population showed less signs of damage in comparison with the type B population (Fig 12) Most of the type A sensory hair bundles were intact and the unilateral orientation was still evident Most of the sensory hairs appeared normal, but in some bundles occasional hairs were fused together (Fig 11) The kinocilia showed no signs of damage Herniating cytoplasmic extrusions were seen at places protruding from the cell surface outside the hair bundle

The dorsal type B population was almost totally destroyed A few distorted hair bundles were seen at intervals (Fig 13) These showed ballooning of the cytoplasm, and sometimes, also dislocation of the cuticular plate The sensory hairs appeared lax, and pointed in all directions The rest of the organ surface was covered by a dense mat of microvilli emanating from the supporting cells

DISCUSSION

Sensory hair deformities in the inner ear organs seems to be the common pattern of reaction to damaging outer stimuli, as well as hereditary disorders Changes in the mammalian inner ear due to exposure to aminoglycoside antibiotics are prone to give a characteristic degenerative pattern in the sensory hairs (Duvall & Wersall, 1963, Lundquist & Wersall, 1967, Wersall et al, 1970 Wersall et al, 1971) Acoustic trauma also induces characteristic sensory hair degeneration (Spoendlin 1958 1962, 1971, Engstrom & Ades, 1960 Lim & Melnik, 1971) Hereditary degeneration of the sensory hairs in the waltzing guinea pig were described by Ernstsson et al (1969)

All the investigations mentioned above were carried out on the mammalian inner-ear Mammals maintain a fixed body temperature and keep a steady high metabolic rate Reptiles are cold-blooded animals where the metabolic rate is dependent on the surrounding temperature In general, reptiles are considered to have a slower metabolism than mammals The hearing organ, the basilar papilla, of the lizard was chosen in order to map the consecutive phases in the development of the sensory hair degeneration The environmental temperature was fixed in order to maintain a milieu which was as near normal as possible No reports on antibiotic metabolism in the reptile have appeared, but one may assume that gentamicin is excreted in the kidney by glomerular filtration, as in the case of mammals Gentamicin sulphate was used in the study (Schering GMC-2-M-205), 1 72 g of gentamicin sulphate corresponded to 1 g of gentamicin base Intraperitoneal administration was chosen as the only possible way of giving the drug Intratympanic administration is not possible as the middle ear of lizards is only a recess from the epipharynx The dosage of 100 mg and 150 mg respectively seemed adequate, regarding the general reaction of the lizards About 30–50 percent of the lizards, in different series, died during the injections This high mortality rate did not occur in untreated animals

Animals treated for 7 days showed no significant sensory hair degeneration This has a high correlation with the findings of Lundquist & Wersall (1966) in kanamycin treated guinea pigs, here, rather severe intracellular changes were observed before sensory hair degeneration became evident Animals injected for 14 days showed a moderate surface degeneration in the basilar papilla A striking feature however, was that the two cell populations reacted in different ways to the drug The ventral type A population was relatively intact in comparison with the dorsal type B sensory cells According to the findings of Weiss et al (1974), the ventral sensory cells are stimulated by low tones while the dorsal cells respond to higher tones This would make the degenera

Fig 11 Part of type A sensory area in a severely damaged basilar papilla Note cytoplasmic herniations (*CH*) and fused hair bundles (*FH*) The majority of the sensory hair bundles appear normal ×7800

Fig 12 Type A sensory area in a severely damaged basilar papilla ×1000

Fig 13 Severely damaged type B sensory cells and supporting cells ×1700


FH
CH

tion pattern in the basilar papilla comparable to that of the mammalian cochlea, where the hair cells of the basal coil are more severely damaged than those of the higher situated coils (Ylikoski et al , 1973), Ylikoski, 1975) This has been explained as a possible result of the spiral flow of the endolymph in the direction towards the basal coils The ototoxic drug is believed to be secreted into the endolymphatic space by the vascular stria A rising gradient of the drug concentration in the endolymph would then be found in the lower levels of the cochlea This theory is, however, not applicable to the findings in the basilar papilla, where no such spiral flow of the endolymph can be anticipated Guinea pigs treated with ototoxic antibiotics as well as with atoxyl also showed a different degenerative reaction between inner and outer hair cells at the same level in the cochlea, Anniko and Wersäll (personal communication) In the severely damaged basilar papilla after 21 days of administration of gentamicin, the degenerative pattern was even more evident The dorsal cell population was now more or less [illegible], while the ventral sensory cells [illegible]e relatively intact

Gentamicin is believed to alter the membrane permeability and maybe also the metabolism in the affected cell (Wersall et al , 1971) Supporting cells of the inner ear, sensory epithelia are far less susceptible to gentamicin treatment than sensory cells This may be due to the difference in metabolic rate between the two types of cells It may also explain the difference in reaction between the two sensory cell populations in the basilar papilla The ventral cells respond to low tones and may have a lower metabolic rate than the dorsal cells responsible for registration of higher tones The dorsal type B sensory cells also have significantly taller hairs, which gives these cells a larger cell surface directly exposed to the endolymph This could increase the resorptive powers of these cells, and thus result in higher intracellular concentration of the drug in these cells This theory could explain the difference in reactive pattern between sensory cells in the same organ The different damage pattern in the two sensory cell populations may also be due to the fact that the ventral type A cells are covered by a tectorial membrane, whereas the dorsal type cells are uncovered The tectorial membrane is anchored to the basilar papilla by a fine meshwork of filaments This structure totally surrounds each hair bundle and creates a tube like formation around the bundle This arrangement may act as a protective barrier against the action of the ototoxic agent Wersall et al (1971) analysed the various stages in sensory hair fusion in the guinea pig crista ampullaris, with the aid of TEM and SEM (1971) SEM alone does not allow a fine structural analysis of the sensory hair changes in single cells On the other hand, it gives an excellent view of the whole basilar papilla, and thus enables a three dimensional study of the pattern of degeneration over the whole surface The formation of giant hairs due to the fusion of hairs as described by Duvall & Wersall (1963) and Wersall et al (1971), is certainly one reason for the protrusion of irregular cytoplasm from the cell surface On the other hand, protrusions were also found consisting of herniated cytoplasm appearing outside the hair bundle as such Even this type of changes seems to be dependent on cell changes caused by the antibiotic, as they are not observed in the normal basilar papilla The cause of the hair changes and the cytoplasma protrusions may depend on direct antibiotic effects on the plasma membrane A secondary damage, due to severe damages of cellular organelles which are essential for the preservation of the plasma membrane is also possible Of special interest with regard to possible direct effects on the plasma membrane is the hair fusion study by Wersall et al (1971) and the study by Schacht (1974) on the effects on the phospholipids of the plasma membrane An analysis of cytoplasmic organelles in damaged cells is in progress and will be presented in a subsequent article

ZUSAMMENFASSUNG

Gentamycin wurde in einer Dosierung von 100 mg und in einigen Fallen von 150 mg per kg Korpergewicht und Tag gesunden Eidechsen der Species *Calotes versicolor* i p verabreicht Die Tiere wurden 7 14 und 21 Tage lang behandelt Nach abgeschlossener Injektionsbehandlung wurden die Tiere getotet und das Hororgan die Papilla basilaris fur die Untersuchung mit dem Rasterelektronenmikroskop aufgearbeitet

Die Tiere zeigten nach 7tagiger Behandlung keine signifikante Oberflachenschädigung der Papilla basilaris Wenn Gentamycin 14 Tage lang verabreicht wurde ging das normale Aussehen der Oberflachenstruktur verloren Die ventralen (apikalen) Typ A Zellen waren verhaltnis massig intakt wahrend die Typ B Zellpopulation in dem dorsalen (basalen) Anteil des Organs Fusionen der sensorischen Haare und zytoplasmatische Herniationen auf wies Die Eidechsen die 21 Tage lang behandelt worden waren zeigten eine stark geschadigte Papilla basilaris Die ventralen (apikalen) Typ A Zellen waren nur massig geschadigt und zwar in Form von Haarfusionen und zytoplasmatischen Herniationen wahrend die dorsalen (basalen) Type B Zellen mehr oder minder zerstort waren Nur gelegentlich waren Zellen erhalten und einige von diesen waren stark geschadigt Die Oberflache des dorsalen (basalen) Teiles des Organs war stattdessen von Stutzzellen bedeckt die eine Art von Narbenstadium darstellten

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AMINOTRANSFERASES IN POST-MORTEM COCHLEAR FLUIDS

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Abstract Aspartate and alanine aminotransferase (ASAT ALAT) activities were measured in human post mortem sera cerebrospinal fluid (CSF) perilymph and endolymph Due to heart and/or liver morbidity during a terminal illness the ASAT and ALAT serum activities were considerably increased as compared with normal and both were 20–30 times higher ($p<0\ 001$) than in CSF or inner ear fluids CSF and inner ear fluids showed mutually similar values

Few data are available on the aspartate and alanine aminotransferases (ASAT and ALAT, formerly known as GOT and GPT, respectively) in the cerebrospinal fluid (CSF) and cochlear fluids Rauch (1964) reported ASAT activ-, in one normal ear to be 7 4 mU/ml and in 10 rotic ears 24 mU/ml, a corresponding average value in 16 guinea pig perilymph samples being 50 mU/ml In CSF, Fleisher et al (1957) reported activity of 15 mU/ml and Bergmeyer (1962) average activity figures of 23–25 mU/ml The perilymph activity figures given by Rauch (1964) for ALAT were 14 mU/ml in 2 non-otosclerotic and 12 mU/ml in 7 otosclerotic ears Bergmeyer's tables showed an average value of 36 mU/ml in CSF As for serum, activity values less than 40 mU/ml are considered normal measured at 37°C, the upper limit at 25°C is 20 mU/ml

ASAT activity in tissues like heart, liver, sceletal muscle and kidney is several thousand times higher than in serum, and the same applies to liver and kidney in the case of ALAT In patients suffering from myocardial infarction or liver disease, serum values are highly increased

In this study we have analysed post mortem sera, CSF and cochlear fluid samples to find out the way in which the normal relationships of these enzyme activities change when the serum values are markedly increased during a terminal illness

MATERIAL AND METHODS

A total of 23 temporal bones were removed at autopsies one or (maximally) two days post mortem During this interval the bodies were kept in store rooms at +5°C The technique of obtaining the samples has been described earlier (Palva & Tikanmaki, 1969) Post mortem serum was obtained from the large thoracic veins and CSF from the spinal canal The detailed technique for aminotransferase quantitative determination at 22°C was identical to the one recently described (Palva et al 1975) For post-mortem fluid analyses, CSF was used undiluted, perilymph and endolymph samples were diluted 1 2, and serum diluted 1 20

RESULTS

The average values and their standard deviations in quantitative analyses of post mortem fluids are given in Table I The perilymph specimens as such were always sufficiently large for analyses, but the endolymph samples had to be pooled from up to 10 temporal bones in order to obtain a few quantitative values Both utricular and cochlear endolymph speci

Table I *Comparison of ASAT and ALAT activity in post mortem fluids (mU/ml)*

	ASAT	ALAT
Serum (N=8)	3 360	1 510
S D	2 035	1 590
CSF (N 10)	98	70
S D	57	57
Perilymph (N=23)	124	22
S D	55	15
Endolymph (N=3)	116	25

mens were used in these pools Compared with normal activity (<20 mU/ml) the aminotrans ferase activity was very high in post mortem serum samples, the dispersion was large, how ever The average values were highly significantly larger ($p<0$ 001) than those found in inner ear fluids and in CSF While the averages for the latter two fluid groups varied somewhat the Student s t test revealed no signifi cant differences between cochlear fluids and CSF From the pooled endolymph samples similar activity figures were obtained as from perilymph

COMMENT

It is quite natural that aminotransferases are highly increased in post mortem sera due to the basic disease and to release of enzymes from damaged organs mostly heart and liver during a terminal illness If these enzymes could dif fuse freely into the CSF and inner ear their activity in these fluids should reflect the serum values This is not the case, however, and the activity in serum remains some 20–30 times higher than in CSF or cochlea As there are no appreciable differences between the latter two, the blood–CSF and blood–cochlear barrier ap parently functions quite effectively up to death The small increase in perilymph and cerebrospinal fluid ASAT and ALAT values as compared with normal data may be a result of passive diffusion from the small capillaries around these spaces after death

These data are in agreement with our earlier findings on the activity of various enzymes on cochlear fluids and CSF as compared with serum, studied post mortem (Palva et al , 1973) The former two fluid groups seem to bear a very close similarity to each other as far as organic large molecular substances are con cerned The actual connection between the two fluids, the cochlear aqueduct, forms a natural pathway between these fluids, allowing a passage of particles extending up to the size of erythrocytes (Holden & Schuknecht, 1968, Palva, 1970) and the barrier towards serum in both fluids apparently functions in much the same way Water and inorganic ions, on the other hand, may pass through the capillary walls without hindrance

ZUSAMMENFASSUNG

Die Aktivitat der Aspartat und Alaninaminotransferasen (ASAT ALAT) wurden post mortem in Serum Zerebrospinalflussigkeit Perilymphe und Endolymphe analysiert Wegen der terminalen Herz und Leberschaden waren die Serumwerte viel hoher als die Normalwerte und beide waren 20–30 mal hoher ($p<0$ 001) als die Aktivitat der jeweiligen Zerebrospinalflussigkeit oder Innenohr flussigkeiten Die beiden letztgenannten zeigten die gleichen Aktivitatswerte

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ELEKTROPHORETISCHE UNTERSUCHUNGEN ZUR PROTEINVERTEILUNG IN DER MEERSCHWEINCHEN-PERILYMPHE

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Abstract. Perilymphe und Serum von Meerschweinchen wurden mittels Immun- und Polyakrylamidgel-Elektrophorese untersucht. Die Molekulargewichte der Perilymphproteine wurden durch Elektrophorese in einem linearen Polyakrylamidgelgradienten (3–20%) ermittelt. Die Immun- und Polyakrylamidgel-Elektropherogramme von äquivalenten Mengen Perilymph- und Serumprotein sind weitgehend ähnlich. Der immunologische Nachweis der Perilymphproteine weist darauf hin, daß es sich um Serumproteine handelt. Für eine eindeutige Identifizierung der einzelnen Proteine fehlen monospezifische Antiseren gegen die einzelnen Proteine des Meerschweinchens. Die Molekulargewichtsermittlung zeigt, daß in der Perilymphe auch relativ hochmolekulare Proteine (Molekulargewichtsabschätzung im Bereich von 67000–290000) sind.

Die zahlreichen Angaben über die Proteinverteilung in der Meerschweinchen-Perilymphe sind sehr unterschiedlich (Chevance et al., 1956, Lazzaroni 1956, Beck & Holz, 1965, Chevance & Usui, 1966, Rademacher, 1966, Rauch & Rauch, 1971, Scheibe et al. 1971, 1972, Giebel, 1972). Fast alle bisherigen Ergebnisse wurden mittels Papier-, Zellulose-azetatfolien- oder Immun-Elektrophorese erhalten. Die Papier- und die Zelluloseazetatfolien-Elektrophorese ermöglichen grundsätzlich keine genaueren qualitativen Aussagen, da die mit diesen Methoden erhaltenen Globulinfraktionen Proteingemische sind (Maurer, 1967). Die unterschiedlichen immunelektrophoretischen Ergebnisse sind offensichtlich auf unterschiedliche immunologische Bedingungen und/oder auf unterschiedlich aufgetragene Proteinmengen zurückzuführen (Scheibe et al., 1972). Bisher konnten wir 5 Perilymphproteine immunelektrophoretisch darstellen. Es was zu vermuten, daß bei einer größeren Menge aufgetragenen Gesamtproteins weitere Proteine in der Perilymphe (PL) nachweisbar sind. In der vorliegenden Arbeit wird über die immunelektrophoretische Untersuchung von konzentrierter Meerschweinchen-PL berichtet sowie über vergleichende Untersuchungen von PL und Serum mittels Polyakrylamidgel-Elektrophorese. Von den gelelektrophoretisch getrennten PL-Proteinen werden Angaben über die Molekulargewichte gemacht.

MATERIAL UND METHODEN

Probengewinnung

Die Untersuchungen wurden mit 200–300 g schweren ohrgesunden Meerschweinchen in Äthylurethan-Narkose durchgeführt. Zur Gewinnung blutfreier PL wurden die Tiere intraarteriell mit Ringer-Lösung perfundiert und die PL sofort danach – aus den beiden Schneckenskalen zusammen – entnommen (Scheibe et al., 1972). Die Blutverunreinigung der PL-Proben wurde durch Erythrozytenzählung kontrolliert. Danach wurden die Proben zentrifugiert. Serum wurde aus Mischblut gewonnen.

Es wurden von insgesamt 90 Tieren 6 Sammel-PL (jeweils 200–500 μl) und 3 Sam-

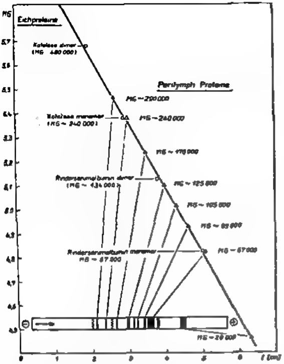

Abb 1 Beziehung zwischen dem Logarithmus (*lg*) des Molekulargewichtes (*MG*) der untersuchten Proteine und deren Wanderungsabschnitt 2 (cm) im Gelgradienten (Fehlerbreite der Methode +5%)

melseren gewonnen Es wurden nur reine PL (Blutverunreinigung unter 0,03%) und hamolysefreies Serum gepoolt Die Protein konzentration der Sammel PL betrug 182–228 mg/100 ml die der Sammelseren 4 250–4 450 mg/100 ml Ein Teil der Sammel PL wurde fur die Immunelektrophorese anfanglich durch Gefriertrocknung, spater mittels Kollodiumhulsen (Sartorius Membranfilter GmbH, Gottingen) im Unterdruck (Krause et al , 1973) maximal 15fach konzentriert (Proteinkonzen tration 2 700–3 000 mg/100 ml)

Proteinbestimmung

Die Proteinbestimmung erfolgte nach Lowry et al (1951) in der schon beschriebenen Mikromodifikation (Scheibe et al , 1975)

Immunelektrophorese

Die Immunelektrophorese wurde in der ebenfalls schon beschriebenen Mikromodifikation (Scheibe et al , 1972) durchgefuhrt Als Antiserum verwendeten wir selbst hergestellte polyspezifische Kaninchen Antiseren gegen Meerschweinchenserum

Polyakrylamidgel Elektrophorese

Die Polyakrylamidgel Elektrophorese (PAA-Gelelektrophorese) mit nachfolgender Molekulargewichtsabschatzung der PL Proteine wurde nach Kopperschlager et al (1969) in einem linearen Gelkonzentrationsgradienten von 3–20% PAA und einem modifizierten Puffersystem (Diezel et al , 1972) durchgefuhrt (Gelpuffer 0 25 M, Imidazol/HCl, pH 7,5 Elektrodenpuffer 0 01 M Imidazol/Diathylbarbitursaure, pH 7 0) Die Elektrophorese wurde bei 110 V, 3 mA/Gelrohrchen und Zimmertemperatur durchgefuhrt und nach 5–6 Stunden beendet Die Farbung er-

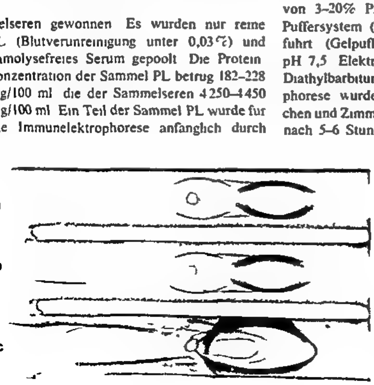

Abb 2 Immunelektropherogramme von je 4 µl PL (*a*) auf die Proteinkonzentration der PL verdunntem Serum (*b*) und unverdunntem Serum (*c*)

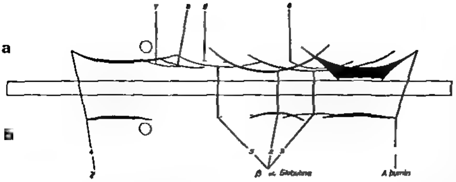

Abb 3 Graph sche Darstellung der Immunelektropherogramme von je 4 μl 15fach konzentnerter (*a*) und normale PL (*b*) D e fortlaufende Nummenerung der nzelnen Praz p ta onsl n en 1 9 entspricht de ab nehmenden Lin en ntens tat

folgte mit Coomassie Brillantblau G 250 Bei diesem Farbstoff besteht eine lineare Beziehung zwischen Proteinmenge und Farbintensitat (Bramhall et al 1969)

Die Molekulargewichtsbestimmung mit Hilfe eines linearen Gelkonzentrationsgradienten beruht darauf daß die Trennung globularer Proteine uberwiegend nach Molekulgroße erfolgt Die Positionen der Proteinbanden im Gel entsprechen daher weitgehend ihren Molekulargewichten Tragt man den Logarithmus des Molekulargewichtes gegen den Wanderungsabschnitt auf so erg bt sich fur die untersuchten globularen Proteine eine Ge

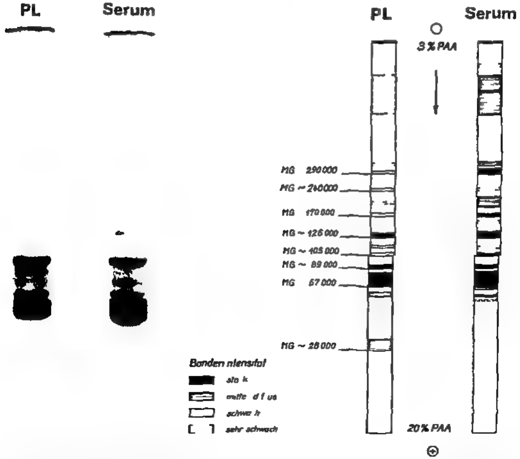

Abb 4 PAA Gelelektropherogramme von PL (100 μl) und Serum (5 μl) m t jewe ls 200 μg aufgetragenem Gesamtprote n n nem l nearen Gelkonzentrationsgrad enten von 3–20% Die ermittelten Molekulargew ch e s nd n der graphischen Darstellung angegeben

rade Wir verwendeten als Eichproteine Rinderserumalbumin (Forschungsinstitut fur Impfstoffe Dessau) und Katalase (EC 1 11 1 6, Rinderleber, Boehringer, Mannheim) in jeweils monomerer und dimerer Form (Abb 1)

ERGEBNISSE

Immunelektrophorese

Typische Immunelektropherogramme von PL (*a*) und Serum (*c*) zeigt die Abb 2 Bei 4 μl reiner PL sind maximal 5 Proteine nachweisbar Bei Serum findet man wesentlich mehr (15–20) und auch starkere Prazipitationslinien, bedingt durch die 20–30 fach hohere Proteinkonzentration des Serums Verdunnt man jedoch Serum auf die Proteinkonzentration der PL (Abb 2*b*) so ergeben sich nahezu gleiche Immunelektropherogramme wie bei PL

Das Ergebnis von der Untersuchung der konzentrierten PL ist in Abb 3 (*a*) zusammengefaßt dargestellt Bei 15fach konzentrierter PL konnten außer den 5 Prazipitationslinien, die auch bei normaler PL auftreten (Abb 3*b*) zusatzlich 4 weitere Prazipitationslinien nachgewiesen werden (Abb 3*a*, Präzipitationslinie 6–9)

Polyakrylamidgel Elektrophorese

Das Ergebnis der gelelektrophoretischen Untersuchungen ist in Abb 4 dargestellt Abb 4 zeigt typische PAA Gelelektropherogramme von PL und Serum mit gleichen Mengen aufgetragenen Gesamtproteins (jeweils 200 μg) Bei dieser Proteinmenge findet man 12–13 Proteinbanden sehr unterschiedlicher Intensitat in der PL und 15–17 im Serum Der großte Teil der beiden Verteilungsmuster ist jedoch hinsichtlich der Bandenpositionen weitgehend ahnlich Die relative Intensitat von entsprechenden Banden der PL und des Serums ist allerdings teilweise unterschiedlich Der hochmolekulare γ Globulinbereich konnte zum Beispiel in der PL nur schwach dargestellt werden Die niedermolekulare Bande (MG ~28 000) trat in der PL mit unterschiedlicher Intensitat auf im Serum war sie nicht nachweisbar Die ermittelten Molekulargewichte der PL Proteine sind in Abb 4 angegeben

DISKUSSION DER ERGEBNISSE

Die immun- und gelelektrophoretischen Untersuchungen zeigen, daß außer den 5 Proteinen, die in normaler PL immunelektrophoretisch nachweisbar sind (Scheibe et al , 1972), weitere Proteine in der PL vorhanden sind Der immunologische Nachweis der PL-Proteine durch Serum Antikorper weist darauf hin, daß es sich um Serumproteine handelt Dafur sprechen auch die weitgehende Ubereinstimmung der PAA Gelelektropherogramme von aquivalenten Mengen PL und Serumprotein Eine Identifizierung der einzelnen Proteine steht noch aus, da fur eine eindeutige Identifizierung monospezifische Antiseren gegen die einzelnen Proteine des Meerschweinchens bisher fehlen Bisherige Angaben uber die Untersuchung der PL Proteine des Meerschweinchens mit der sog Disk Laurell Elektrophorese (Giebel, 1972, Giebel & Saechtling, 1973) weisen ebenfalls darauf hin, daß in der PL die meisten Serumproteine vorhanden sind wenn auch in wesentlich geringerer Konzentration als im Serum

Die gelelektrophoretische Molekulargewichtsbestimmung zeigt daß in der PL auch relativ hochmolekulare Proteine (Molekulargewichtsabschatzung bis 290 000) nachweisbar sind Die Fraktion mit einem Molekulargewicht von ungefähr 125 000 scheint nach Albumin (MG ~67 000) die zweitstarkste Proteinfraktion in der PL zu sein Bisher wird noch die Ansicht vertreten (Schnieder 1974), daß Makromolekule mit einem Molekulargewicht uber 50 000 nicht vom Blut in die PL gelangen Eine Diskussion uber eine Permeabilitats grenze einer sog Blut PL Schranke ist wohl zum gegenwartigen Zeitpunkt verfruht da uber einen Schrankenmechanismus bisher nichts bekannt ist und experimentell gemessene Austauschraten von einzelnen Makromolekulen (Neiger 1968 Schnieder, 1970) nicht ohne weiteres verallgemeinert werden konnen

SUMMARY

Perilymph and serum of guinea pigs were investigated using immunoelectrophoresis and polyacrylamide gel electrophoresis *The molecular weights of the perilymph proteins* were estimated by electrophoresis in a linear polyacrylamide gel gradient (3–20%) The immunoelectropherograms and the polyacrylamide gel electropherograms of equivalent amounts of perilymph protein and serum protein are nearly the same The immunological detection of the perilymph proteins indicates that they are serum proteins The clear identification of the individual proteins fails because of missing monospecific antisera against the individual proteins of guinea pig High molecular proteins (range of the molecular weight estimation 67000–290000) are also detectable in the perilymph according to the molecular weight estimation

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VESTIBULAR EVOKED POTENTIALS IN THALAMUS AND BASAL GANGLIA OF THE SQUIRREL MONKEY *(SAIMIRI SCIUREUS)*

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Abstract In anesthetized squirrel monkeys vestibular representation in the thalamus and basal ganglia was determined by field potential recording using peripheral electrical vestibular nerve stimulation Vestibular thalamic regions were investigated for cortical connections Two relatively large thalamic areas nucleus ventralis posterolateralis VPL and the posterior nuclear group (Po) received vestibular inputs with short latencies suggesting direct connections with the vestibular nuclei Antidromic stimulation of the area $3a$ vestibular field did not produce responses in any of the vestibular thalamic fields The vestibular regions in VPL and Po can be antidromically invaded from SI and the anterior parietal lobe respectively In the striatum vestibular fields were found in the suprathalamic portion of the nucleus caudatus and dorsomedially in the putamen

Peripheral input reaches the neocortex via thalamic neurons The input from the vestibular labyrinth has been located within the primary somatosensory cortical field, SI, for several species and an additional parietal field is known for primates and carnivores (Fredrickson et al 1974 Schwarz & Fredrickson, 1974 for recent reviews) The thalamic cell groups carrying vestibular information to these fields have not been clearly identified although vestibular activity has been described within a variety of thalamic locations (Copack et al 1972 Deecke et al 1973 1974 Hassler 1948 1964 1972 Mickle & Ades 1954 Raymond et al 1974 Sans et al 1970 Spiegel et al , 1965 Wepsic 1966)

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Anatomical methods are not suitable to identify the cells involved, because vestibular cortical fields receive proprioceptive input in addition to the vestibular inflow It is therefore necessary to demonstrate that thalamic neurons projecting to vestibular cortical fields also receive afferents from the labyrinth

The purpose of this study was to demonstrate the thalamic location of the vestibular relay and its cortical connections in the species where the projection to area $3a$ (SI) first was described

MATERIAL AND METHODS

This study is based on two separate series of experiments First, in 18 adult squirrel monkeys vestibular foci were localized in the thalamus An attempt was made to identify the cell population projecting to the vestibular cortical field in area $3a$ Since this was not successful cortical projection of the defined vestibular thalamic regions was later systematically investigated in another 5 monkeys

All experiments were performed under pentobarbital anesthesia (30 mg/kg i p additional doses i v) deep enough to prevent barbiturate spindles The animals were tracheotomized continuously infused with 5% sucrose in lactated Ringer s solution and kept at a body temperature of 37 to 38°C The

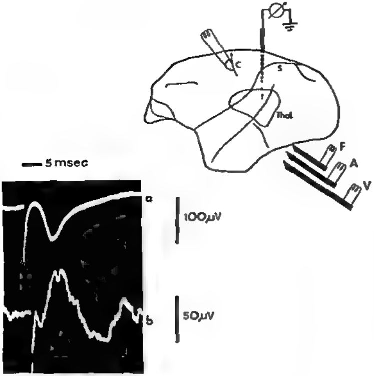

Fig 1 Vestibular field potential recording Diagram of stimulation and recording electrodes V=vestibular nerve A=auditory nerve F=facial nerve C=central sulcus S=Sylvian fissure Specimen recordings *a* vestibular evoked surface potential in the area 3*a* field *b* thalamic vestibular field potential in VPLo Negativity upwards as in all Figs

Thalamic abbreviations

CL=nucleus centralis lateralis
CM=nucleus centrum medianum
sl=nucleus centralis superior lateralis
=nucleus lateralis dorsalis
=nucleus limitans
=nucleus lateralis posterior
ID=nucleus medialis dorsalis
MGmc=corpus geniculatis medialis pars magnocellularis
MGpc=corpus geniculatis medalis pars parvocellularis
Pf=nucleus parafasciculatis
Po=the posterior nuclear group
PO=nucleus posterior
Pt=nucleus parataenialis
Pulv=pulvinar
Ret=nucleus reticularis
Sg=nucleus suprageniculatus
St=subthalamus
VB=the ventrobasilar complex
VI=nucleus ventralis intermedius
VL=nucleus ventralis lateralis
VPI=nucleus ventralis posterior inferior
VPL=nucleus ventralis posterior lateralis
VPLc=the caudal part of the former nucleus
VPLo the oral part of the former nucleus
VPM nucleus ventralis posterior medialis
ZI=zona incerta

arrangement of stimulation electrodes employed is illustrated in Fig 1 Only square wave voltage shocks (0 1 msec 1/sec) were used for stimulation

Isolated stimulation of labyrinthine afferents was achieved via bipolar Ag–AgCl electrodes (120 μm insulated diameter) attached to the anterior branch of the vestibular nerve usually one wire to the lateral canal branch and one to the utricular branch In order to control current spread identical electrodes were positioned on the facial nerve just distal to the ganglion geniculi and on the auditory nerve within the Modiolus These electrode pairs were embedded in paraffin (42°C melting point) to exclude formation of conducting tissue fluid bridges and fixed to the bone with dental cement

Since the external ear canal was destroyed because of this surgical lateral approach only one ear could be used for fixation of the head in the stereotaxic frame On the operated side a brass plate cemented to the occiput was therefore firmly screwed to the frame The contralateral cerebral cortex was exposed by craniotomy to allow access to the thalamus

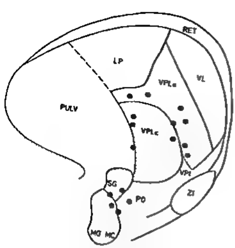

Fig. 2. Vestibular thalamic foci. Each dot indicates the center of a typical focus in this composite diagram.

with the recording micro pipettes and to the area $3a$ vestibular field. After the latter had been identified by evoked potential recording a bipolar silver electrode (tip separation 2 mm) was inserted into the cortex until a negative potential was recorded from one of these wires. All thalamic sites yielding vestibular responses were tested for antidromic potentials using stimuli to this cortical electrode pair. Glass micro pipettes (ca. 1 MΩ, 2M NaCl) were used for thalamic field potential recording. In each experiment several electrode tracks were positioned in one plane (usually in the sagittal, occasionally in the frontal direction). Conventional a.c. recording (0.1 Hz–300 Hz) was employed and specimen recordings were photographed from an oscilloscope. Since in most cases field potentials were of small and background activity of high amplitude the clear demonstration of a vestibular focus frequently depended on computer averaging (PDP8e, summation of 25 sweeps, binwidth 0.1 or 1 msec for 100 bins).

Cortical projection of vestibular thalamic areas was investigated in 5 additional monkeys using the same anesthesia and recording equipment. The movable cortical surface stimulation electrode consisted of two steel balls with a separation of 1.5 mm. The cortical region from which a thalamic antidromic field potential could be recorded was thus determined and recorded on a cortical map. In order to facilitate thalamic location during recording, thalamic field potentials to the following peripheral stimuli were also determined: intracutaneous electrical shocks to the wrist and ankle areas of the limbs. Recordings were obtained at 0.5 mm steps. Thalamic field potentials on cortical stimulation were interpreted to be antidromic when the latency was short (1 msec ±0.5 msec) and neither latency nor amplitude changed when the stimulation rate was altered from 1/sec to 100/sec at stimulus intensities above threshold but well below maximum for the amplitude of the field potential.

After the recording session all animals were sacrificed by perfusion with a 10% formaldehyde solution. The brains were fixed, dehydrated, embedded in celloidine and cut in the plane of the electrode tracks in sections of 100 μm which were stained with cresylviolet. All recording positions were reconstructed and drawn on a composite thalamic diagram.

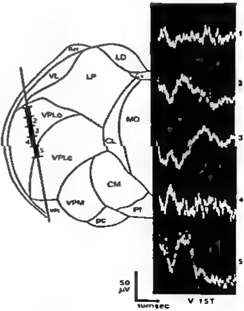

Fig. 3. Specimen recordings along an electrode track through VPLo. Note polarity reversal of the early deflection. V 1.5 *T*=Vestibular stimulus at 1.5 times threshold intensity.

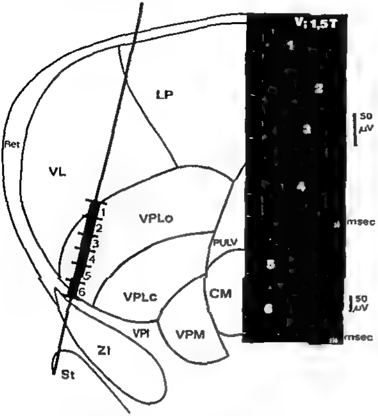

Fig 4 Averaged field potentials along an electrode track in VPLo. Vestibular latency is 2.5 msec. Note different time base for recordings 1–4 and 5–6 (10 and 100 msec respectively)

using a microprojector. Tissue shrinkage was -timated by comparing the original electrode nces with those in the histological slides alamic nuclei were labelled according to Olszewski's atlas for the Rhesus monkey (1952).

RESULTS

Vestibular field potentials were recorded during 116 electrode penetrations through thalamus and basal ganglia in [illegible] squirrel monkeys. Recording was generally difficult since amplitudes were small enough to be masked by background activity (cf. Figs 3, 5). Therefore computer averaging was usually necessary. It might be objected that widely dispersed potentials with low amplitudes hint at the possibility of volume conduction from a distant common potential source (current sink for negative deflections). This was excluded by a systematic laminar field analysis recording at 100–200 μm distance throughout each electrode track (Figs 3–6) and using several tracks for each of the vestibular foci. Vestibular field potentials with latencies shorter than the cortical evoked potential were observed to

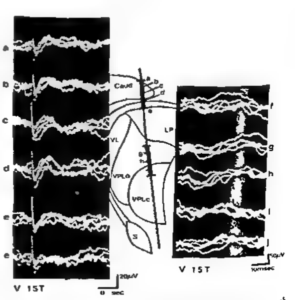

Fig 5 Specimen recordings along an electrode track passing through nucleus caudatus and the dorsal part of VPLo

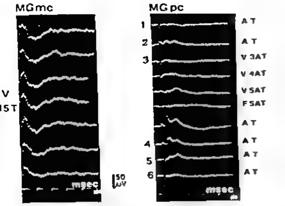

Fig 6 Averaged vestibular field potentials in MGmc and auditory field potentials in MGpc at series of electrode positions 100 µm apart Note that the MGpc potential with high intensity vestibular nerve stimulation (V 5AT 5 times threshold value for auditory nerve) matches the time course of the auditory rather than the vestibular potential in MGmc

be generated in foci dispersed over two thalamic regions the VPL and the posterior nuclear group (Po) (Fig 2)

Vestibular fields in VPL

The VPL has been subdivided into an oral (VPLo) and caudal (VPLc) division on cyto architectonic criteria (Olszewski, 1952) The oral part VPLo (=nucleus ventralis interme dius VI, of C Vogt 1909) contains the largest thalamic cells and is known to receive muscle afferents This partition is particularly large in the squirrel monkey and extends dorsally over VPL wedging caudally between LP and the pulvinar

Most of the vestibular field potentials were found in this division Occasionally a polarity reversal along subsequent recording sites of an electrode track was seen (Fig 3) In most potential series, however there was a maxi mal negative amplitude at one position simply attenuating in either direction (Figs 4 5) It is not reasonable to assume systematically dif ferent field characteristics in a structure which is morphologically as uniform as VPLo Ap parently current sinks tend to be more concen trated and are thus easier recorded whereas sources are wider dispersed, reversal would only be recorded when the electrode travels along long neuronal elements (e g long den drites) giving rise to both current sink and source

Vestibular fields were not concentrated at one location within the large VPLo but rather dispersed throughout its extent This implies that all regions of VPLo are equally involved in the transmission of vestibular and other afferents (e g from muscle spindles) A bipartition of VPLo (VI) in a medial vestibular and lateral proprioceptive zone (Hassler, 1972) can accordingly not be assumed for Saimiri A more functional interpretation of the input to this nucleus is possible on the basis of a neuronal analysis (Liedgren et al , in prep)

As seen in Fig 2, also the caudal border zone of VPLc yielded vestibular field poten tials This region has been known to receive input from joint receptors (Poggio & Mount castle, 1960, 1963) Within the anterior and central portions of VPLc, however, no ves tibular fields could be identified

Latencies of vestibular field potentials in both VPLo and VPLc were always shorter (2 5 to 3 msec) than those of the cortical evoked potential (ca 4 msec) in area 3*a* However, evidently all these VPL fields do not transmit vestibular information towards the small area 3*a* vestibular field in a convergent fashion In fact, isolated stimulation of this field was in no case sufficient to evoke antidromic field poten tials at vestibular foci In a subsequent series of 5 experiments designed to map out cortical projection of the thalamic vestibular fields, it was, of course, easy to evoke antidromic field potentials throughout VPL in the known topo graphical order with stimuli to SI including area 3*a* Taken together these data suggest a vestibular projection to wider areas of the ventrobasilar complex, VB, and SI blending into its somatotopic arrangement The small vestibular area 3*a* field as defined by evoked potential recording demonstrates only a small portion of a more diffuse projection system

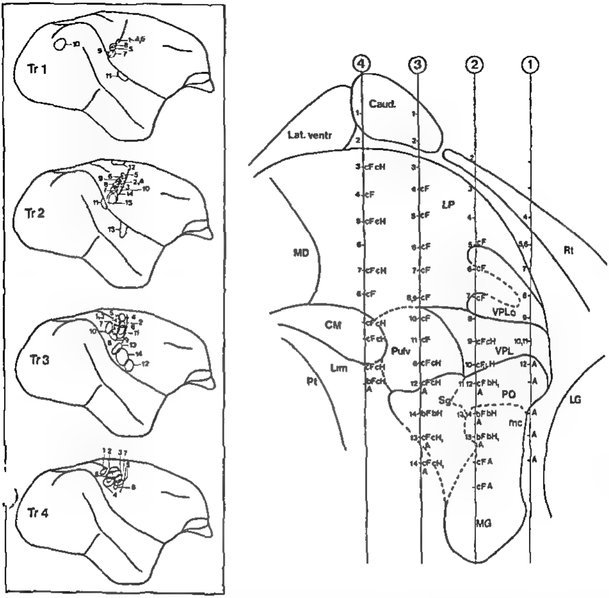

Fig. 7. Cortical origin of antidromic thalamic field potentials. Diagram of a histological reconstruction of four electrode tracks in a coronal plane. Cortical maps 1–4 correspond to track numbers 1–4. Recording sites are 0.5 mm apart. To the left of each site the number of the corresponding cortical projection field (cf: cortical map) is indicated, to the right the peripheral input as determined by standard field potential recording. *c*=contralateral, *i*=ipsilateral, *b*=bilateral, *F*=forelimb, *H*=hindlimb, *A* auditory nerve.

Posterior nuclear group

Most vestibular fields within these nuclei were found in the MGmc (Fig. 2), although also PO and Sg appear to receive labyrinthine information. A more recent unitary analysis supports this projection for all three nuclei (Liedgren et al., in prep.). In Fig. 6 vestibular field potentials in MGmc are contrasted with auditory potentials in MGpc. It is clear that isolated stimulation of the vestibular nerve can evoke field potentials almost throughout the entire depth of the magnocellular portion whereas in the parvocellular portion intensities must be raised to values sufficient for current spread to

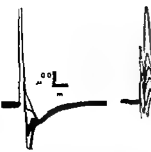

Fig 8 Antidromic field potentials recorded in the LP as the stimulation electrode is slided from the border of the cortical projective field (superimposed recordings to the right) into its centre (*left*) The maximum negative potential within the centre (*left*) does not change latency or amplitude when stimulus frequency is changed from 1/sec to 100/sec

the cochlear nerve before vestibular potentials are seen (Fig 6 position 3 V 5AT) Current spread from the facial nerve to the cochlear nerve did not occur even at this high intensity (Fig 6 position 3 F 5AT) It was of course not difficult to evoke MGpc potentials with cochlear nerve stimulation at low intensities (latency ca 5 msec)

Vestibular latencies in the Po were comparable with those in the VPL A projection to the area 3*a* field can however be excluded Antidromic field potentials were not evoked from the SI area in MGmc PO and Sg but rather from the rostral region of the parietal cortex (Fig 7) A few parietal locations (Tr 2 14 15 Tr 3 13 14) giving rise to antidromic field potentials in MGmc and adjacent structures may correspond to the parietal vestibular field found in the macaque i e the parietal region bordering the transition zone between hand and face districts of SI (Fredrickson et al 1966) Antidromic field potentials in Po can however also be evoked from other stimulation sites in the anterior parietal lobe A vestibulo parietal projection may be too diffuse and/or asynchronous to be detected using evoked potential recording in anesthetized squirrel monkeys The data available suggest however such a projection to be carried via MGmc and adjacent cell groups

Vestibular field potentials with longer latencies

It is evident in Fig 7 that a topographically organized parietal projection originates in LP Vestibular information may be transported via this route although connections are probably less direct The earliest vestibular evoked potentials in LP had a latency of 5 msec Similar potentials were found in the pulvinar in the CM as well as in the suprathalamic portion of the caudate nucleus (Fig 5) and adjacent medio-dorsal portions of the putamen

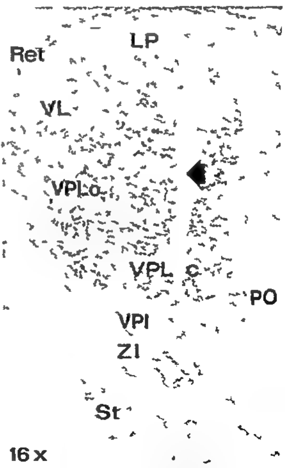

Fig 9 Lesion caused by an electrode left with a recording position during fixation and dehydration The track passes through the dorsal portion of VPLo before entering VPLc Vestibular field potentials were recorded at the position marked by the arrow

DISCUSSION

The main purpose of this study was to localize thalamic stations of the vestibulo cortical pathway in the only primate species for which a vestibular projection to area 3*a* is known (Odkvist et al, 1974) A second cortical field in the parietal lobe, homologous to that in the Rhesus (Fredrickson et al, 1966, Schwarz & Fredrickson, 1971) could not be demonstrated for Saimiri in an earlier evoked potential study It is however, unreasonable to doubt corresponding connections The results of the present study suggest that relatively direct vestibular information reaches both SI and the parietal lobe Proof for the former projection is available in a recent single unit study (Liedgren et al, in prep), whereas the latter assumption is supported by conjectural evidence provided here

In view of the small vestibular cortical field it is surprising that so wide thalamic areas within two separate nuclear zones were found to receive vestibular input with bi-to trisynaptic latencies Considering the small amplitudes and the difficulties in demonstrating thalamic vestibular foci it is not probable that the complete cortical projection can be discovered by means of evoked potential recording Cortical vestibular fields defined on basis of evoked potentials in anesthetized animals can apparently only illustrate a part of this projection

A small isolated projection field in area 3*a* would point at a confinement of vestibular inflow to a small cluster of cells within the VB complex A vestibular representation in VB exists, but it appears to be rather dispersed over almost the total extent of the large VPLo and the caudal border zone of VPLc VPLo corresponds to nucleus intermedius (VI) in Vogt's terminology (1909) and is known to receive group I muscle afferents (Andersson et al, 1966, Goto et al, 1968, Jasper & Bertrand 1966, Landgren & Silfvenius, 1970, Mallart, 1964, 1968, Rosen, 1969) Caudally in VPLc a kinesthetic representation has been described (Poggio & Mountcastle, 1960, 1963) The vestibular inflow thus appears to be directed to the regions where various aspects of movement and position sense are represented

Hassler (1964, 1972) has described a direct connection between vestibular nuclei and the medial portion of VI (VPLo) in the cat The forelimb is represented medially in VPLo and hindlimb afferents project to its lateral portion (Andersson et al, 1966, Landgren & Silfvenius, 1970) The medial VPLo zone appears to fit the cortical vestibular projection to the forelimb field of area 3*a*, possibly the vestibular inflow to the forelimb region is just concentrated and synchronized enough to permit cortical evoked potential recording with gross electrodes, whereas the vestibular afferents within other partitions of the proprioceptive system are too widely dispersed to be demonstrable at the cortical level with a technique of such a poor resolution power With regard to this it is not surprising that antidromic stimulation of the small area 3*a* field could not unveil the thalamo cortical connections this projection is directed to larger portions of SI and originates in wide thalamic areas A vestibular projection to VPL has been reported for the cat using both electrophysiological (Sans et al, 1970) and anatomical (Raymond et al 1974) techniques In these studies, the cytoarchitectonic identity of VPLo (VI) was not recognized, therefore no analogies with the data presented here are possible

A second thalamic region, Po, receiving relatively direct labyrinthine input includes MGmc, Sg and the posterior nucleus (PO) Vestibular field potentials in this area have previously been recorded in the cat (Copack et al, 1972) and Rhesus monkey (Deecke et al 1973, 1974) The short latency potentials in the Rhesus were however, interpreted as fiber activity being carried further rostrally It is evident from recent unitary recordings (Liedgren et al, in prep) that cells in these nuclei can be excited by vestibular nerve stimulation with latencies as short as 2 5 msec Many neurons in MGmc, Sg and PO receive

converging input from vestibular receptors and from large and often bilateral somatosensory RF s and/or the cochlea The traditional claim that this type of sensory input is unspecific merely illustrates the general ignorance concerning the function of these nuclei The data now available suggest that vestibular input is transmitted from this region to the parietal lobe, which is thought to serve higher and thus more specific functions in the hierarchical arrangement of sensory analysis

In the cat anterograde degeneration following lesions in the posterior nuclei was found in a fringe zone around the somatosensory and auditory fields including the suprasylvian vestibular field (Graybiel, 1973) Part of this region is homologous to the primates parietal lobe If a parietal vestibular field is assumed for Saimiri with the same location as in the macaque it should be situated just caudally to the border zone between hand and face representation of SI The MGmc projection fields 2 14, 15 and 3 13, 14 in Fig 7 would satisfactorily match such an assumption However, vestibular representation in Po is dispersed over wide regions as in VPL Accordingly it appears reasonable to assume a more diffuse distribution of vestibular inflow to the anterior parietal lobe Our antidromic cortical maps demonstrate that MGmc and Sg project to the parietal region bordering SI posteriorly This does not imply, however that all anterior parietal vestibular activity must originate from Po also LP projects to this zone and vestibular potentials with a longer latency (5 msec) were observed in this nucleus

The vestibular input to the Rhesus parietal field was previously assumed to originate in VPI (Deecke et al 1973 1974) In the present field potential study we were unable to provide evidence for either a vestibular input to VPI or a parietal projection of this nucleus Considering the difficulties involved in demonstrating vestibular field potentials in the thalamus exclusion of any cell group as a possible thalamic vestibular station should be referred to a more sensitive unitary analysis

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Die vestibulare Projektion in Thalamus und Basalganglien wurde bei Totenkopfäffchen bestimmt indem Feldpotentiale bei elektrischer Reizung des Vestibularisnerven abgeleitet wurden Thalamische vestibulare Felder wurden dann auf kortikale Verbindungen hin überprüft Zwei relativ grosse thalamische Kerngruppen empfangen vestibulare Afferenzen mit so kurzen Latenzen dass eine direkte Verbindung mit Vestibulariskernen angenommen werden kann VPL und die posteriore Kerngruppe (Po) Elektrische Reizung des vestibularen Feldes in Area 3*a* reichte nicht aus um antidrome Potentiale in thalamischen Vestibularisfeldern abzuleiten Vestibulare Regionen in VPL konnen von SI aus antidrom aktiviert werden die in Po vom rostralen Parietallappen Vestibulare Felder im Striatum wurden im suprathalamischen Teil des Nucleus caudatus und dorso-medial im Putamen lokalisiert

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INTERACTION BETWEEN THE SACCULES AND THE VERTICAL SEMICIRCULAR CANALS

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Abstract For the experiments 39 cats were used Selective sectioning of either the anterior or the posterior vertical ampullar nerves was performed bilaterally Additionally in some cats the utricular nerves were cut or the saccule extirpated on one or both sides The otolith organs were stimulated by tilting around the bitemporal axis of the animals Bilateral sectioning of the anterior ampullar nerve induced vertical nystagmus upwards which disappeared if the animals were tilted upside down When the posterior ampullar nerves were cut bilaterally a vertical nystagmus downwards was obtained and if the animals were tilted upside down there was a large increase in nystagmus frequency After unilateral extirpation of the saccule vertical nystagmus downwards was observed in 50 percent of these experiments This nystagmus was modulated by tilting However if the saccules on both sides were extirpated no nystagmus could be elicited The cause of these results is discussed

Opinions regarding the function of the saccule have differed widely In 1883 Sewall opened the saccule of the ray with a knife and found that this caused serious disturbances in the animal s equilibrium Later Versteegh (1927) was unable to observe any changes in the vestibular reflexes after destruction of both saccules in the rabbit He therefore suggested that the saccule has no vestibular function Other investigators have attributed hearing functions to the saccule Ashcroft & Hallpike (1934) obtained action potentials from the saccular nerve after subjecting a frog s labyrinth to low frequency vibrations Von Bekesy (1935) observed, after exposing human subjects to loud sound beats head movements that were synchronous with the beats He states that this was caused by a stimulation of the saccular otoliths Jongkees (1950) rejected however, the idea of a cochlear function and suggested that sounds of low frequency might be regarded as rapidly changing linear accelerations, which stimulate the saccule On the basis of morphological investigations, Lorente de Nò (1933) maintained that an acoustic function of the saccule cannot be accepted, because it has no connection with the auditory pathways and centres

Adrian (1942) demonstrated, that if the saccular otoliths were stimulated by linear acceleration, the activity in the vestibular nuclei changed Subsequently, several other authors have shown that the saccule reacts to linear acceleration (Benjamins & Huizinga, 1927, Huizinga 1955, Jongkees 1950, Loewenstein, 1950, Magnus & de Kleyn, 1926, Perlman, 1940 Szentagothai, 1952)

One of the first to observe the saccular influence on oculomotor reactions was Kubo (1906) He stimulated the saccule in the shark with a piece of cotton which resulted in distinct eye movements Quix (1924) considered that the rotating eye movements, in animals with their eyes in a lateral position, were released from the utricle whereas Magnus & de Kleyn (1926) thought that these reactions originated from the saccule Szentagothai (1952) demonstrated on dogs distinct eye movements after mechanical stimula

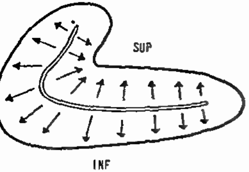


Fig 1 Schemat c diagram of the anatomical orientat on of the two saccular areas on the left s de

tion of the otolith membrane in the saccule Huizinga (1955) and Benjamins & Huizinga (1927 1928) observed that counter rolling of the eyes in pigeons disappeared after destruc tion of the saccule Jongkees (1950) reported that rabbits react normally to thermal and rotatory stimuli after both unilateral and bilat eral destruction of the saccules Nor was Janeke (1968) able to find any alteration in the labyrinth responses provoked by rotation around a cephalocaudal axis in rabbits Con sequently he concluded that the function of the ule is overshadowed by the intact utricle & Shizu (1960) resected the saccular in rabbits and found that the com atory deviation of the eyeball was influ enced by the saccule

In recent years it has become much easier to understand the function of the saccule as more knowledge of the morphology of this organ has been acquired Loewenstein et al (1964) have shown that the saccular surface can be divided into two areas depending on the orientation of the kinocilia and the stereocilia (Fig 1) In the superior area the kinocilia are oriented upwards in relation to the stereocilia and in the inferior area down wards

It is known that these sensory cells are stimulated when the stereocilia bend towards the kinocilia (Loewenstein & Wersall 1959) Fluur & Mellstrom (1970) demonstrated that it was possible by electrical stimulation of the inferior area to release eye movements downwards If the superior area was stimu lated they observed eye movements upwards and in some experiments even vertical ny stagmus directed downwards In earlier ex periments Fluur & Siegborn (1973*a b c d* 1974*a b*) investigated the interaction between the utricles and the semicircular canals and demonstrated a very intimate cooperation be tween these sensory receptors

Thus the aim of this investigation has been to find out if the same relation exists between the saccule and the vertical semicircular ca nals and whether the saccule as such is able to induce nystagmus

EXPERIMENTAL PRINCIPLES

As previously mentioned electrical stimulation of the two areas of the saccule releases distinct eye movements upwards or downwards Therefore it was decided to study the saccular influence on the vertical semicircular canals Accordingly either the anterior or the pos terior vertical ampullar nerve was selectively sectioned bilaterally The superior area of the saccule was stimulated by tilting the animals upside down around their bitemporal axis In some experiments the utricular interference was eliminated by sectioning the utricular nerves bilaterally In other experiments the vestibular balance was disturbed by extirpa tion of the saccule on one or both sides

MATERIALS AND METHODS

For the experiments 39 healthy cats were used After intubation during ether anesthesia the cats were given Ketamine (Ketalar® Parke Davis) during the operation and the recording of eye movements Ketamine is a nonbarbituric anesthetic with pronounced analgesic properties and a minimal effect on the vestibulo-ocular reflexes (Fluur & Siegborn 1974*c*) The investigation was di vided into four parts

1 Selective sectioning of either the anterior or the posterior vertical ampullar nerves com

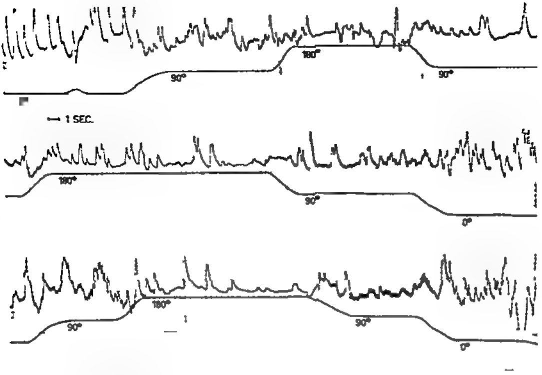


Fig 2 Consecutive curves from a cat after selective bilateral section of the anterior ampullar nerves followed by tilting upside down The upper curve shows electronystagmography and the lower curve tilting of the table

bined with sectioning of the utricular nerves followed by tilting the animal upside down around its bitemporal axis

II Selective sectioning of the anterior vertical ampullar nerves followed by tilting the animal upside down around its bitemporal axis

III Selective sectioning of the posterior vertical ampullar nerves followed by tilting the animal upside down around its bitemporal axis

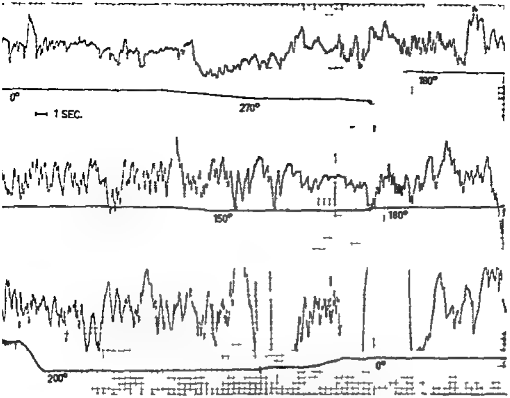
IV Unilateral and bilateral extirpation of the saccule followed by tilting the animal upside down around its bitemporal axis

When sectioning the anterior ampullar nerve or the utricular nerve a small fenestration was made above the oval window according to a method described earlier by Fluur & Siegborn (1973*a* *d*) For the sectioning of the posterior ampullar nerve the bulla was explored and a fenestration was made behind the round window according to the method described by Fluur & Siegborn (1974*a*) As it is almost impossible to selectively section the saccular nerve the saccule was extirpated through the oval window The animals were operated on a tilting table where it was afterwards possible to stimulate the otolith organs by tilting the table all round the animals bitemporal axis The angle of tilting was measured in degrees where 0° was the normal position 90° the nose up position, 180° the upside-down position and 270° the nose-down position

The eye movements were studied by electronystagmographical recordings with electrodes above and below the eye and by visual inspection

RESULTS

Selective sectioning of either anterior or posterior vertical ampullar nerves, combined with sectioning of utricular nerves followed by



g 3 Consecut ve curves from a cat after select ve lateral sect on ng of the posterior ampullar nerves llowed by tilting ups de down The upper curve shows electronystagmography and the lower curve t lt ng of the table

ting the animal upside down around its lateral axis

nitially both the anterior ampullar and the tricular nerves were sectioned bilaterally in 4 ats This resulted in a very weak nystagmus pwards which was totally inhibited if the nimals were tilted upside down In another eries the posterior ampullar and the utricular erves were cut bilaterally in 5 animals which esulted in a total disappearance of nystagmus ' they were tilted upside down nystagmus ownwards was developed Because of the eak nystagmus that occurred in these series e following experiments included only sec oning of the vertical ampullar nerves

elective sectioning of anterior vertical am ullar nerves followed by tilting the animal pside down around its bitemporal axis

fter selective sectioning of the anterior mpullar nerves 8 cats presented vertical nystagmus upwards The animals were then tilted to the 90° position around the bitemporal axis which caused an increase in nystagmus frequency whereas a decrease amounting almost to a total inhibition was observed in the 270° position If the cats were then tilted from the nose up position to the 180° position the nystagmus was totally inhibited This in hibitive action on nystagmus frequency was observed already at the 135° position How ever if the animals were tilted forwards to the *270° position the nystagmus was inhibited* and remained so until the 180° position was passed (Fig 2)

Selective sectioning of posterior vertical am pullar nerves followed by tilting the animal upside down around its bitemporal axis

At the next stage of the experiment the posterior ampullar nerves were sectioned bilaterally in 8 cats whereafter a weak vertical

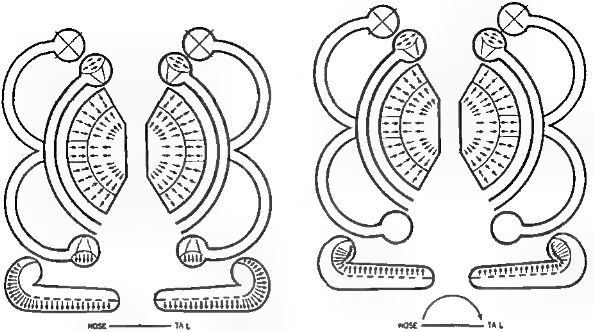


Fig 4 Schematic diagram of the labyrinth after bilateral selective sectioning of the anterior ampullar nerves in resting position and when the animal is tilted upside down The arrows indicate the orientation of the cells i e the direction in which they increase the discharge frequency The number of arrows symbolizes discharge frequency

nystagmus downwards occurred When the animals were tilted to the 90° position the nystagmus frequency decreased in 5 cats, and nystagmus was totally inhibited in 3 cats On tilting the animals further backwards around the bitemporal axis, nystagmus reappeared successively after the 135° position was passed, and increased to a maximum, when the cats were in the upside-down position On the other hand, if they were tilted forwards the nystagmus increased, and reached a maximum when the table was at the 210° position Tilting from an initial position of 180° to the 150° position resulted in a slight decrease in nystagmus intensity Tilting, in the opposite direction to the 210° position caused an increase in nystagmus frequency (Fig 3)

Unilateral and bilateral extirpation of the saccule followed by tilting the animal upside down around its bitemporal axis

In 14 cats the saccule was extirpated on one side A vertical nystagmus downwards was observed in 7 animals, which increased in the upside down position, while in the other 7 no nystagmus was detectable in any position In the cats with nystagmus, an increase was also seen at the 270° position, whereas a reduction in nystagmus frequency was observed in the nose-up position Then the other saccule was also extirpated, which resulted in a total disappearance of nystagmus, which it was impossible to induce again by tilting

DISCUSSION

In order to eliminate the influence from the utricles during tilting, the utricular nerves were cut in addition to bilateral sectioning of either the anterior or the posterior ampullar nerves In these experiments no nystagmus or only a weak nystagmus developed, probably because the intensity of the impulses from the periphery were too low to trigger the nystagmus reflex This seems especially to have been

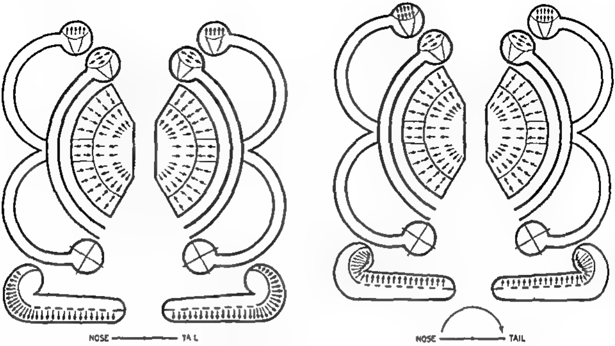


Fig 5 Schematic diagram of the labyrinth after bilateral selective sectioning of the posterior ampullar nerves in resting position and when the animal is tilted upside down Arrow indication the same as in Fig 4 The number of arrows symbolizes discharge frequency

the case after sectioning the posterior ampullar and the utricular nerves This could be due er to diminished total input from the tibular organs or to the fact that the tivity from the remaining anterior vertical micircular canals was antagonistic to the activity from the inferior saccular area, when the cats were in the normal position From this stage of the experiment it may be concluded *that, even in the absence of the utricles the* saccules are able to influence the activity from the vertical semicircular canals so that a nystagmus induced by the latter can be changed However if only the anterior vertical ampullar nerves were cut a vertical nystagmus upwards occurred as a result of the activity from the posterior vertical semicircular canals When the animals were tilted upside down the nystagmus was totally inhibited In this position the superior saccular area, which causes eye movements upwards is stimulated Thus, the activity released from this area is antagonistic to the activity from the posterior semicircular canals (Fig 4)

There was also a combined influence on the activity from the posterior vertical semicircular canals from both the utricles and the saccules which was demonstrated at the 150° and the 210° positions

At the next stage of this investigation the posterior, vertical ampullar nerves were sectioned bilaterally, and vertical nystagmus downwards was seen, which was due to the *activity from the remaining anterior semicircular canals* When these animals were tilted upside down the nystagmus intensity increased This could be explained by the fact that the anterior vertical semicircular canals release eye movements upwards, i e in the same direction as those from the superior saccular area Thus, these activities are synergistic in their effects on the extra ocular muscles (Fig 5) At the 150° and the 210° position a combined activity of the saccules and the utricles on the activity from the anterior vertical semicircular canals was demonstrated

Finally, at the last stage of this investigation

the saccule was extirpated unilaterally, which in 50 percent of the cases induced a nystagmus downwards Although the activities of the two saccules were synergistic, this unilateral destruction must have caused a disturbance in the balance between the two vestibulo-ocular reflex arcs, which induces nystagmus However, a bilateral extirpation of the saccules does not seem to have disturbed the vestibular activity These results are in accordance with those obtained by Fluur & Mellstrom (1970) They reported that in some cases, when electrically stimulating the superior area of the saccule they occasionally observed vertical nystagmus downwards

Thus, the results of this investigation have shown that, in principle, the saccule, as such, can not induce nystagmus, but this might be elicited by unilateral saccular damage

When studying the interaction between the saccules and the vertical semicircular canals, only the superior saccular area was stimulated, because it is not possible to stimulate the inferior area without relatively complicated equipment, since this has to be stimulated with forces exceeding 1 *g* in the direction of the sensory cells in this area

The results of these experiments lead to the general conclusion that the saccular activity modulates the impulses released from either the anterior or the posterior, vertical semicircular canals Moreover, these results show that, like the utricles, the saccules play an important integrative role together with the vertical semicircular canals in the vestibular system

ZUSAMMENFASSUNG

Fur die Versuche wurden 39 Katzen verwendet Eine selektive Durchtrennung der anterioren oder posterioren vertikalen Ampullennerven wurde beidseitig durchgefuhrt Zusatzlich wurden bei einigen Katzen die Utricularnerven oder der Sacculus auf einer Seite oder auf beiden Seiten entfernt Die Stimulierung der Otolithen organe erfolgte durch Neigung der Tiere in der bitemporalen Achse Bilaterale Durchschneidung der anterioren Ampullennerven induzierte einen vertikalen Nystagmus nach oben der aufhorte wenn die Tiere mit der Unterseite nach oben geneigt wurden Wenn der posteriore Ampullennerv beidseitig abgetrennt wurde erfolgte ein vertikaler Nystagmus nach unten, und bei Neigung der Tiere mit der Unterseite nach oben kam es zu einer starken Zunahme der Nystagmusfrequenz Nach unilateraler Entfernung des Sacculus wurde bei 50% der Versuche ein vertikaler Nystagmus nach unten beobachtet Dieser Nystagmus konnte durch Kippung moduliert werden Wurde jedoch der Sacculus auf beiden Seiten entfernt konnte kein Nystagmus erzeugt werden Der kausale Zusammenhang dieser Ergebnisse wird diskutiert

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ACTIVITY OF SINGLE MEDIAL GENICULATE UNITS IN RESPONSE TO SINGLE AND DOUBLE CLICKS

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Abstract The discharge pattern to clicks and the time course and pattern of the click induced inhibition in the medial geniculate body have been studied by single unit recording technique The medial geniculate neurones showed a tendency to fire at preferred latencies after a click A conditioning click caused a reduced number and increased latency of the discharges to a subsequent test click Provided conditioning click of a certain strength was employed the inhibition was often cyclic, each period lasting for about 100–150 ms Clicks could also trigger barbiturate spindles with synchrony between the activity of the medial geniculate body and the primary auditory cortex Interaction experiments suggest that the triggered and spontaneous spindle activity operate with the same neurones

The medial geniculate (MG) neurones undergo prolonged inhibition in response to a click (Aitkin & Dunlop, 1968, Etholm, 1975) This inhibition alters the discharge of MG neurones Such an alteration may take the form of changes in discharge pattern latency, or probability of discharge Apart from latency reduction to clicks of increasing strength and the presence of click-induced rhythmic discharges of MG neurones (Galambos et al 1952, Aitkin et al 1966, Aitkin & Dunlop, 1968), relatively little is known about the finer details of the firing pattern of MG cells and how this may be changed by inhibition

The purpose of the present investigation has, therefore, been to study how the inhibition at the medial geniculate level is able to change the discharge pattern of single units An additional purpose was to study whether the inhibition can be correlated with the tendency to rhythmic discharge of the medial geniculate neurones

This investigation has been supported by the Norwegian Research Council for Science and the Humanities and the University of Oslo

METHODS

The methods have been described in detail in the preceding paper (Etholm, 1975) Briefly, unitary discharges of the medial geniculate neurones were recorded with glass micro-electrodes, filled with 4 M NaCl and having resistances of 5–20 MΩ Clicks (main frequency 620 Hz) were used both as conditioning and test stimuli The click amplitude was damped to 10% of the peak value within 11 ms In addition to the short latency responses to clicks, continuous and click induced rhythmic activity was recorded from the MG body and the auditory cortex and displayed either on an oscilloscope and filmed, or on an ink writer

RESULTS

Pattern of discharge

The analysis concentrated upon MG cells with a latency to the first discharge between 7 and 10 ms which most likely represent rela cells

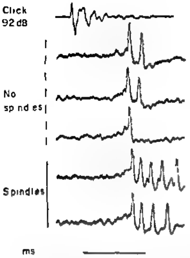


Fig 1 Click evoked (92 dB) medial geniculate unit responses Upper trace gives the microphone record Second to fourth trace show responses with two and one spikes Fifth and sixth traces show responses with spindles

(Aitkin & Dunlop, 1968) Under the chosen experimental conditions a characteristic feature of the discharges of medial geniculate units was a certain degree of variability (Fig 1) The upper trace gives the microphone [illegible]d of the click, whereas the next traces [illegible] consecutive micro-electrode records [illegible] cell discharged one or two spikes with [illegible]tial latency of about [illegible] ms or fired a high-frequency burst with nearly the same initial latency (lower two records) The tendency to discharge in such bursts was clearly correlated to the spontaneous rhythmic barbiturate spindle discharges This variability had to be taken into account when studying the tendency to multiple spike discharges The difficulty could largely be circumvented by rejecting data taken simultaneously with spontaneous spindle activity or by studying a large number of records Both methods were used

Another source of interference was the background noise The experiments were performed in a general neurophysiological laboratory where the noise level was around 45 dB for three peaks at 50, 90 and 180 Hz, but only 30–32 dB at 500 Hz, close to the main frequency of the click In particular, loud voices or traffic noise could be seen to interfere with the spike discharges, usually by increasing their latency, but sometimes also by blocking the discharges altogether Therefore, the most reliable data were obtained in the evenings or at night

A common feature of the discharge pattern of MG cells is a tendency to discharge more than one spike This tendency is illustrated in Fig 2B, in which many superimposed records show that the cells tended to discharge twice,

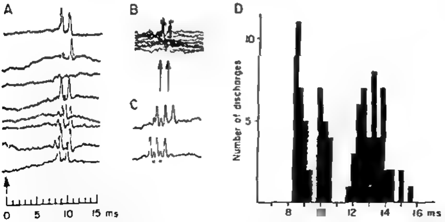


Fig 2 Medial geniculate unit answers in response to a click (A) Consecutive records which are to be read from down upwards The cell most often discharged twice (B) Many superimposed records The cell tended to discharge twice about two preferred latencies (C) Two cells—one small with a tendency to discharge twice whereas the other (large spikes) discharged three times (D) A post stimulus histogram with spikes occurring at preferred times and with one cluster around 8 5 ms and another around 10 ms

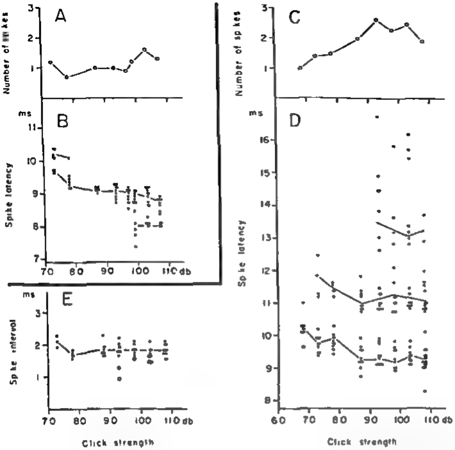


Fig 3 Shows MG unit answers in response to clicks of increasing strength (A) Number of spikes (B) Spike latency Note the clustering about preferred times (C) Another experiment showing the number of spikes (D) Spike latency and clustering around three preferred times (E) Spike intervals which tend to occur with an interval of 1 8 ms The encircled data indicate spike intervals when the discharge had more than four units

and that the time of the discharges were centering around two preferred latencies In Fig 2C, are two other cells, one small cell (filled circles) tended to discharge twice, whereas another cell (large spikes) discharged three times As evident from the records, the time of discharge could vary, but not randomly The unitary discharges tended to occur around given value after the onset of the click This can also be seen in Fig 2A where consecutive records (to be read from below upwards) show that the cells most often discharged twice, and that these discharges tended to occur at a fixed interspike interval Occasionally, the cell showed only one discharge but this usually occurred at a latency corresponding to one of the discharges in the double firing This tendency to a double or triple discharge, with spikes occurring at preferred times, is also seen by the post stimulus diagrams, one example of which is given in Fig 2D

In response to clicks of increasing strength, the MG unitary discharges showed a systematic behaviour. Increasing strength gave an increased number of spikes and reduced latency However, the latency reduction to gradual increase of the click was not gradual, but occurred in definite steps Fig 3B shows a cell which at weak clicks (73 dB) discharged simply, either around 9 6 or around 10 2 ms latency At 78 dB, however, the cell tended to

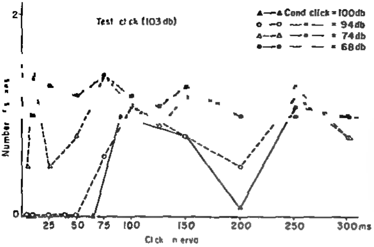


Fig 4 Effect of conditioning clicks of increasing strength on the number of spikes evoked by a 103 dB test click delivered at various intervals after a conditioning click The two strongest conditioning clicks caused a profound inhibition of a cyclic nature

discharge only at the first preferred time, which now was reduced to about 9 3 ms With further increase, the cell discharged frequently at the earliest time, which now was from 8 8 to 9 2 ms When the click was increased to 98 dB the cell usually discharged twice, the first spike being recruited at an even earlier latency with the second spike occurring at the same time as the single discharge to weaker clicks (Fig 3 A) These times were not absolutely ed, but showed slight variations around a an value

Fig 3 D shows the behaviour of a cell which tended to discharge several spikes in response to a click With weak clicks the latency of discharge varied between 9 5 and 11 ms with a clustering around 10 2 ms On increasing the strength the initial latency was reduced so that from 87 to 108 dB the mean latency measured 9 2–9 3 ms The second spike also had a tendency to occur within certain time limits, roughly between 11 and 12 ms A similar trend was seen for the third spike in the train which, although less frequent tended to occur between 13 and 14 ms

The relatively constant interspike interval is plotted in Fig 3 E The spike tended to occur with an interval of 1 8 ms The encircled data indicate spike intervals when the discharge consisted of more than 4 units This was associated with the occurrence of spindles which probably leads to burst discharge through a different mechanism than that produced by orthodromical impulses from clicks

Degree and time course of inhibition of spike discharges

Click induced inhibition of single units in the MG body was observed by Aitkin & Dunlop (1968), and confirmed in the present series A click had an inhibitory effect on MG spike discharge as tested by a subsequent test click At appropriate test intervals, this appeared as a reduced number of spike discharges or as an increased latency of discharge The inhibition was dependent upon the strength of the conditioning click In the experiment shown in Fig 4 the intensity of the test click was kept at 103 dB while the strength of the conditioning click was varied between 68 and 100 dB The abscissa represents the interval between the conditioning and test clicks and the ordinate gives the average number of spikes per trial in response to the test click The shading indicates the range of variation of the number of spike discharges (1 0–1 6) produced by the test stimulus alone With a conditioning click intensity of 68 dB the number of spikes of the test response did not change at all (●) By increasing the strength of the conditioning click to 74 dB (Δ) there was a clear inhibition as shown by a reduced number of discharges

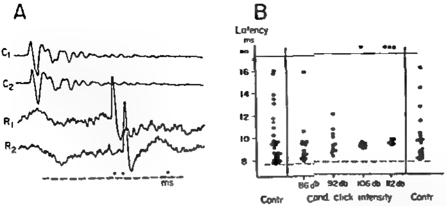


Fig. 5. (A, B) Increase in latency of a single MG unit neuronal discharge in response to a test click (C 1 and C 2) caused by a conditioning click of varying strength delivered 30 ms before the test click. R 1 and R 2 giving the unconditioned and conditioned (112 dB) response of the MG neurone respectively. Conditioning caused a latency increase of 1.3 ms. (B) Discharge latencies of a single MG unit in response to a 110 dB test click are expressed along the ordinate in the columns to the left and right. The central four columns give the discharge latency of the same neurone as altered by four conditioning clicks at the indicated strengths. O: failures.

at 25 and 50 ms intervals (mean of 10 observations). At an interval of 10 ms, the spike number was normal, probably due to summation of the excitatory effects of the conditioning and test clicks. The reduced response at an interval of 5 ms can most likely be explained by refractoriness or occlusion of the activity in auditory nuclei below the medial geniculate level caused by the two stimuli. At an interval of 75 ms, the responses again were within the normal range. Increasing the conditioning click intensity to 94 and later to 100 dB, gave a more pronounced inhibition (O, ▲ respectively). At both of these conditioning intensities, the inhibition was total at click intervals from 5 to 50 ms. At an interval of 200 ms, there was a second inhibitory effect, particularly with the strongest conditioning click (100 dB). This cyclic inhibition was commonly, but not invariably observed. It occurred in experiments with spontaneous rhythmic spindle activity.

Another indicator of inhibition was an increased latency of the cellular discharges. As for the number of discharges, both the duration and degree of the latency increase were dependent upon the strength of the conditioning click. In Fig. 5A the R 1 response was produced by an unconditioned test click (C 1) whereas R 2 followed a test click (C 2) conditioned by a preceding click. The conditioned response (R 2) showed an increase of the discharge latency. In order to establish that such latency increases were not due to spontaneous variability, a number of unit discharges had to be plotted. In Fig. 5B, the left and right hand columns (Contr.) show the discharge latencies of this particular unit

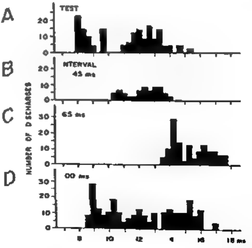


Fig. 6. Post stimulus histogram. (A) Response to a click of 104 dB. (B, C, D) Response with a preceding conditioning click at the given intervals. *Abscissa*: Response latency. *Ordinate*: Number of discharges.

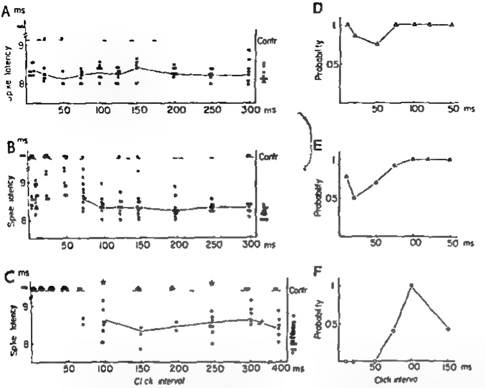


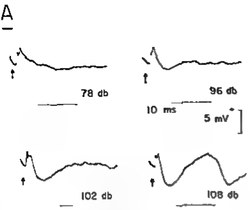
Fig 7 Time course of the increased latency and the probability of a single MG unit caused by conditioning clicks of varying strength (A D) Results when the conditioning click intensity was [illegible] dB (B [illegible] 74 dB (C F) 94 dB The stars above the stippled line (C) indicate no failures at click intervals of 100 and 250 ms

ich only fired once on each trial The cell wed the described tendency to discharge preferred times, either at latencies around 8–8 5 or around 9 5 ms

When a conditioning click of 86 dB was delivered 30 ms before the test shock, the late discharges were reduced in number but the clustering of the discharges around the mentioned latencies were maintained The same applies when the conditioning click was increased to 92 dB, but the latency of the clusters increased (broken line gives shortest control latency) At conditioning click intensities of 106 and 112 dB, the discharges occurred in one cluster only and with a clearly longer latency than the controls Another interesting feature in this situation was the markedly reduced variability of the discharge and the occurrence of failures (O)

A similar change in the pattern of discharge and increased latency was seen when the conditioning click was delivered at various times before the test click Fig 6 A shows the post stimulus histogram of the responses to a click of 104 dB The tendency to grouped occurrence is evident When a click of 110 dB preceded the test click by 45 ms (B), the latency increased but the peaked appearance was maintained At an interval of 65 ms the latency was further increased, and at an interval of 100 ms the latency seemed to be similar to the control again Thus, the inhibitory effect of the transmission through MG will not only result in a reduced transmission but those impulses that are led through, occur at the cortex at a later time than normal

The time course of the latency effect is illustrated in Fig 7 A–C In each graph the abscissa represents the interval between the conditioning and test clicks The ordinate gives the spike latency in ms The test click, whose intensity remained at 104 dB through



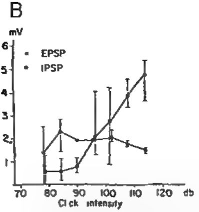


Fig 8 (A) Quasi intracellular recording from a cell in the parvocellular part of MG Responses to increasing strengths indicated Note the cyclic IPSPs response to a click of 108 dB (B) Size of EPSP and IPSP recorded from the same neurone in response to increasing click intensities

out this experiment caused the cell to discharge with an initial latency around 8 2 ms At a conditioning intensity of 68 dB (A), no clear effect could be observed on the spike latency However, at click intervals of 25 and 50 ms there were cases of failure (A D) Such failures did not occur in the control records When the conditioning strength was increased to 74 dB (B) there was an increase of spike latency as well as a considerable number of failures (E) at conditioning test click intervals below 100 ms The increase of failures was even more marked when the conditioning click intensity was increased to 94 dB (C), when complete failure occurred at intervals of 50 ms and below (F) At intervals of 100 ms and 250 ms, however, there were no failures indicating a reduction of the inhibition at these times (*)

Latency increases were seen in all relay cells studied for this purpose

The observation that similar latency in

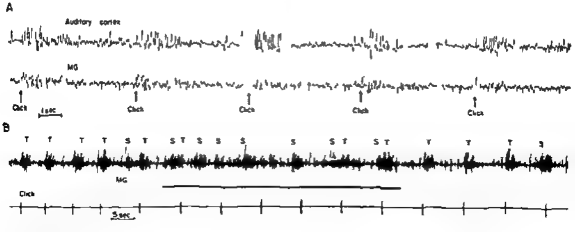


Fig 9 Spontaneous (*S*) and triggered (*T*) spindles in the primary auditory cortex (upper trace) and the medial geniculate body (lower trace) (A) Sequence of triggered spindles taken at medium speed on the ink writer Clicks indicated by arrows Note correspondence between the spindles in the two recording stations (B) Similar to (A) but recorded on slower time base The lower trace of each pair marks the delivery of the clicks

crease or failure of transmission was observed following stimulation either of the cortex or of the white matter, suggests that part of the mechanism is due to a recurrent inhibition, similar to that already established for the somatosensory thalamo cortical system (Andersen et al, 1964*b*)

Postsynaptic inhibitory potentials (IPSPs)

The parvocellular part of the MG body proved a difficult target for intracellular recording The small size of the cells combined with the relatively firm connective tissue overlying the dorsal and caudal part of the thalamus made it difficult to obtain good intracellular recordings These experiences were similar to those of Nelson & Erulkar (1963)

Fig 8 shows some examples from a MG cell (upper trace) The arrow gives the onset of the click A weak click (78 dB) produced a depolarizing potential on top of which small local responses are seen It is likely that the underlying depolarizing potential can be classified as an excitatory postsynaptic potential (EPSP) By gradual increase of the click the EPSP urred suddenly, showing a *unitary* charac r) Further increase of the click gave no clear ncrease of the size of the EPSP (Fig 6B)

Following the EPSP there was a hyperpolarizing response, which grew in size as the click intensity was increased Since this hyperpolarizing response also could occur without a preceding spike discharge and was associated with cessation of spontaneous discharges, it is likely that this deflection represents an inhibitory postsynaptic potential (IPSP)

The amplitude of the EPSP in relation to increasing click strength was remarkably different from that of the IPSP In Fig 6B is shown how the EPSP once appeared increased very little while the IPSP after a short initial period showed a gradual and steady rise One possible explanation for this phenomenon is that relatively few axons converge on the relay neurone to produce an EPSP composed of a few unitary EPSPs The IPSP, however, is probably due to recruitment of an increasing number of inhibitory interneurones and possibly also an increasing duration of their respective discharges This pattern would be compatible with a forward as well as a recurrent type of inhibition Since typical IPSPs with similar growing amplitude also were seen in response to antidromic stimulation, recurrent inhibition seems a likely possibility

Sometimes, as seen by the record labelled 108 dB in Fig 6A, single click produced two sequential IPSPs, similar to the repetitive IPSPs recorded from various other thalamic systems under moderate barbiturate anaesthesia (Andersen et al, 1964*a* McIlwain & Creutzfeldt, 1967) The circumstances under which repeated IPSPs to single stimuli were seen, were clearly associated with moderately light barbiturate anaesthesia in which spontaneous spindle activity was present

Triggered and spontaneous rhythmic activity of MG neurones

Rhythmic 10/second activity in other thalamic nuclei, either triggered by a synchronous afferent volley or occurring spontaneously has been associated with spontaneous barbiturate cortical spindles (Andersen & Andersson 1968) Since a single shock under appropriate circumstances was able to elicit a series of IPSPs in MG cells, it was of interest to see whether a single shock also could trigger spindle discharges, both in the medial geniculate body and in the appropriate cortical projection area In Fig 9 the upper trace shows a surface recording from the A I part of the auditory cortex The lower trace shows the activity obtained simultaneously by a micro electrode located in the medial geniculate body In order to compare the slow activity of the two sites the micro-electrode recording was high frequency filtered (3 dB down at 50 Hz) and displayed on an ink writer Clearly both the MG and the auditory cortical area had

a tendency to rhythmic activity This was particularly evident in response to the clicks Each click set off a series of waves in the medial geniculate body which had their direct counterpart in the spindle activity of the auditory cortex The elicited rhythmic activity was, however, not simply due to repetitive bursts discharges in the way of the slow after-discharges of Bremer & Bonnet (1950) since the deflections were not damped, but showed initial augmentation The sequences are, therefore, probably triggered spindles

The correspondence, and at times competition, between the triggered and the spontaneous spindle activity were evident (Fig 9B) Each triggered spindle is indicated by the letter T, whereas spontaneous spindles are indicated by S The marks on the lower line indicate the time of delivery of clicks The first four spindles were triggered by the click When a spontaneous spindle occurred within a critical period before a test click such as the series of 7 spontaneous spindles marked by the horizontal bar, the triggered spindle response to a click was markedly diminished On the other hand, if the spindles were triggered more frequently as if forestalling the next spontaneous spindle, the system did not seem available to the spontaneous "pacemaker"

With a repetition rate above a certain critical frequency, which varied with the depth of anaesthesia but often was around one every 5 seconds, it was possible to drive the spindle activity consistently with a click Such triggered spindles would then be obtained even if the interval between two subsequent spindles was further reduced However, there was a minimal interval for successful triggering of spindles This interval changed somewhat from situation to situation but was usually about twice the duration of the spindle itself If the clicks were delivered more frequently than the minimal interval the triggering ability was markedly reduced Also when a click was delivered too soon after a spontaneous spindle, the spindle response to the click was reduced or even abolished

DISCUSSION

Preferred time of firing

A striking finding was the tendency of mec geniculate relay neurones to fire at prefer intervals after a click Although spontane latency variations occurred, partly caused autochthonous central nervous activity a partly by the varying background noise in experimental room, the discharges fell wit distinct time-limits This held true also cells firing more than one spike Each of spikes in a group showed a distinct tende to occur at a given latency An increase of stimulus intensity caused only a small, grad reduction in the spike latency until a sudd quantal jump in latency occurred This haviour could be explained by preservatior the pattern of repetitive discharge correspo ing to the click frequency which has b found in the primary auditory nerve fib (Kiang et al , 1962) In some cases this ex nation seems likely because there was a go correspondence between the main freque of the click and the spacing of the neuro discharges in the burst (Fig 1 A, lower trac However, in other circumstances, the f quency of the multiple discharge of the M cell differed from the main frequency of click Fig 2 is an example where the inter between the discharges was only 0 6 to 1 0 whereas the main frequency of the click w 620 Hz corresponding to a 1 6 ms period T possibility that such discharges occur alternative peaks of the click induced volle also unlikely since the jump always took pl between two fixed preferred latencies and to latencies half way between these two v ues Therefore for this pattern it is proba that a transformation taking place in audit nuclei beyond the primary auditory fibres least in the geniculate body itself, may responsible for the repetitive discharges It therefore, possible that the multiple dischar in the MG are due to a double mechanism T first is likely due to a temporal coding of click as described by Kiang et al (1962) and

Rupert et al, (1963) The other mechanism operating in this context is probably largely residing inside the medial geniculate body and may be explained by converging nerve volleys which are slightly dispersed in time Summation of large but slightly asynchronous EPSPs would give multi peaked summed excitatory synaptic potentials which could explain the observed repetitive behaviour of the unit discharges corresponding to the referred times of firing Such peaks have indeed been recorded in some MG cells (Nelson & Erulkar, 1963)

The disappearance of the spikes with longest latencies at high click intensities may be explained by the occurrence of an IPSP which curtails the excitatory effect

The studies of single MG unit behaviour to clicks of increasing intensity showed a remarkably sharp threshold for spike discharge in most cells suggesting that few but large synapses are brought into action This observation is in accordance with the large glomerular synapses in this part of the auditory nervous system (Majorossy & Rethelyi, 1968) and also helps in explaining the steep input/output curves of the auditory system

Inhibitory processes in MG body

The input/output curve for the IPSPs of MG cells differed from that of the EPSPs suggesting that the IPSP received contribution from many elements, supporting the proposal of an interneuronal inhibitory system for the MG cells as proposed by Aitkin & Dunlop (1968) In analogy with the findings in the somato sensory system (Andersen & Eccles, 1962) the inhibition may be recurrent since inhibition could be induced by antidromic stimulation This interpretation is also supported by the tendency to cyclic inhibition (Figs 4, 8) and the rebound burst discharges between two subsequent inhibitory periods On the basis of the present experiments it is not possible to decide whether forward inhibition also contributes to the inhibition in MG

Triggered and spontaneous spindle activity

There was a remarkable similarity in configuration and appearance between the spontaneous spindles in MG and the auditory cortex and those triggered by a click Because the system could be driven either by a spontaneous 'pacemaker' or by a synchronous afferent volley produced by the click, the system is probably operating with the same neuronal elements in both the 'triggered' and the 'spontaneous" mode In this respect, the behaviour of the medial geniculate neurons is similar to that of ventro basal cells (Andersen & Andersson 1968) The MG spindles are probably due to the same general mechanism involving postsynaptic inhibition and post inhibitory rebound discharges like those observed in response to clicks in the present investigation

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ZUSAMMENFASSUNG

Das Entladungsmuster von Klicksen und ihrer Latenzzeit und das Muster der klicksinduzierten Inhibition im Corpus geniculatum mediale wurden mit einer Technik untersucht die das Registrieren von Einzelzellen erlaubt Die Neuronen im Corpus geniculatum mediale zeigten die Tendenz in bestimmten Zeitabständen nach einem Klicks loszuschiessen Ein auslosender Klicks und ein nachfolgender Test Klicks verursachten eine verminderte Anzahl und eine erhohte Latenzzeit der Entladung einer Zelle Vorausgesetzt dass eine gewisse Starke des auslosenden Klicks benutzt wurde verlief die Inhibition oft zyklisch in Perioden die ungefähr 100–150 ms dauerten Die Klicks konnten auch Barbituratspindeln in Gang setzen wobei Übereinstimmung zwischen der Aktivität im Corpus geniculatum mediale und der auditorischen Cortex bestand Die Experimente zeigten auch dass die durch Klickse in Gang gesetzten und die spontanen Spindeln mit denselben Neuronen arbeiteten

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SIZE AND DURATION OF INHIBITION IN THE MEDIAL GENICULATE BODY IN UNANESTHETIZED CATS

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Abstract A method which allows repeated micro elec trode recordings from subcortical structures without using any drugs is described This method was adopted in combination with convential implantation techniques to study click evoked potentials and inhibitory processes in the auditory system of the cat The click evoked poten tials in MG were hardly affected by moderate doses of barbiturate and only to a minor degree in the auditory cortex In the unanaesthetized animal the most significant contribution to the click evoked inhibition in the auditory system was due to mechanisms in the MG The inhibition was diminished both in size and duration as compared with the situation in anaesthetized cats The MG cells showed a tendency to cyclic inhibition in the unanaes thetized cat but not so regularly as following administration of sodium pentobarbital The action of barbiturates on the auditory system is discussed

In the ventrobasal complex of the thalamus Poggio & Mountcastle (1963) demonstrated a longer inhibition in anaesthetized monkeys than in unanaesthetized, but flaxedilazed monkeys with previous bilateral transaction of the trigeminal nerves and ascending branches of the cervical plexus Aitkin et al (1966) reported similar results when comparing me dial geniculate (MG) responses from flax-edilized and barbiturized cats Kitahata et al (1969) found corresponding data for halothane and barbiturate anaesthetized animals In the present experiments the upper levels of the auditory system have been studied in waking or sleeping cats with repeated micro electrode recordings, employing a method devised by Gjerstad & Skrede (1970) This method allows experiments without use of drugs The data were later correlated with results obtained from the same cats, but in barbiturate anaes thesia It was found that the evoked potential in MG was hardly depressed by the barbiturate anaesthesia The inhibition of the MG poten tials caused by a preceding click, however, were greatly prolonged

METHODS

Preoperative training

Six adult cats of both sexes, selected for tameness, were used During the week prior to the operation the cats received their single daily meal on the recording platform By gentle handling and stroking they were persuaded to stay on the platform After a few days' training they often fell asleep and could stay on the recording platform for several hours

Operation and recovery

The cats were anaesthetized with sodium pentobarbital, 40 mg/kg intraperitoneally Un der aseptic condition the skull was carefully cleaned of muscles and connective tissue a circular hole of diameter 20 mm was drilled in the bone, leaving the dura intact The centre of the hole was placed over the sagittal sinus A polyvinyl chloride (PVC) cylinder (Fig 1B c)

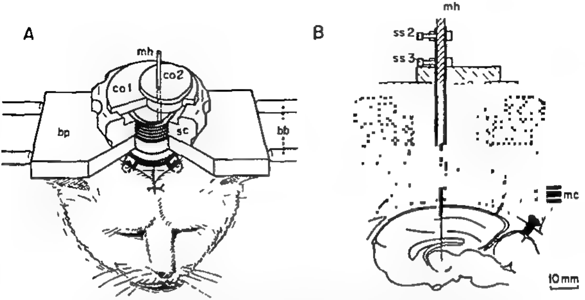


Fig. 1. Diagram showing the arrangement for fixing the head of the cat to the animal frame. (*B*) Diagram showing a sagittal section through the brain and skull of the animal with the fastening arrangement above. Abbreviations: *bb*: brass plate, *co 1*: large cover, *co 2*, inner cover, *cy*: plastic cylinder, *gu*: electrode guide with a coarse cannula at its lower end for penetration of the dura, *mc*, microcontact, *mh*: microelectrode holder, *ss* 1, 2, 3: set screws, *sc*: screw cap.

with an inner diameter of 20 mm, was placed over the hole and affixed with dental acrylic and stainless steel screws. 10–12 cortical electrodes were implanted in the precruciate and auditory cortices. Combined recordings from the "killed ends" of the thalamo-cortical fibres and the auditory cortex were obtained by pushing the electrode through the cortex and about 5 mm into the white matter. The recording electrodes were made of silver or stainless steel wires, insulated except for 1 mm at the tip. The animals were grounded via one of the screws in the skull (Fig. 1B). The leads were connected to a micro-connector (Fig. 1B, *mc*) secured with dental acrylic to the PVC cylinder and the occipital bone.

The cats were left to recover for about one week after surgery. During this time, and later on, when no recording was performed, the PVC cylinder was filled with sterile Ringer's solution, and sealed with a perspex plug. The cats were fed on the recording platform only, but were not fixed to the holder.

Fixation and postoperative training

The cats were fixed by the head-cylinder to a 10 mm thick brass plate (Fig. 1A, *bp*) with 4 brass bars (*bb*) which were held in an animal frame (head holder). The PVC cylinder fitted into a circular hole in the brass plate. A PVC ring (Fig. 1, *sc*), screwed on to the PVC cylinder, kept the cat's head from moving in the vertical plane, and a set screw (Fig. 1B, *ss* 1) on the brass plate counteracted rotation.

Most cats initially refused to be fastened, and one to two weeks of active training were usually required to get the cats accustomed to the situation. The cats were fastened to the animal frame for increasing periods of time, starting with a few minutes. After some weeks the cats were quiet and relaxed for several hours. During this period the cats were fed exclusively on the platform in the recording room, usually as a reward for having been fastened. The length of the training periods varied, depending on the tameness of the cat. One cat was regarded as impossible to train,

and therefore excluded after a trial of about 3 weeks At the other end of the scale another cat jumped by itself on to the recording platform after 2 days of training only lay down and started purring The cats be haviour while secured to the frame indicated that they had no physical pain After being properly trained they rapidly lay down often began to purr and soon fell asleep They frequently accepted milk from a syringe It was possible to keep each of our successful cats (five) fastened for 10–12 hours during an experiment without the cats showing signs of discomfort If during an experiment a cat showed signs of discomfort or incipient dis tress as judged from increased respiration frequency dilation of the pupils miaowing or scratching it was immediately released

Micro electrode recording

Micro-electrode recordings were performed from one week to maximally 4 months post operatively After the animals were secured to the animal frame the perspex plug was re moved the Ringer solution replaced by erilized liquid paraffin and the cylinder ered by two circular transparent perspex vers (Fig 1 *co* 1 and *co* ?) fitting into a circular groove in the PVC ring (Fig 1 *sc*) The smaller perspex cover (*co* 2) was situated excentrically inside the bigger one having a hole that fitted exactly to the guide (Fig 1B *gu*) of the micro-electrode holder (Fig 1B *mh*) This hole was again excentrically placed in the small cover making it possible to rotate the two covers with respect to each other to reach all points within the cylinder with the micro-electrode (Evarts 1968) Thus a closed system minimizing the brain movements was attained

The micro electrodes were made from tung sten wire (0 5 mm) electrolytically tapered to a tip diameter of 1–2 mm and insulated with lacquer except at the tip (Hubel 1957) The micro electrode was fixed to the micro-elec trode holder but insulated from it The micro electrode holder (Fig 1 *mh*) consisted of a stainless steel tube (outer diameter 2 5 mm) which could slide inside a stainless steel guide (Fig 1B *gu*) The guide could be fastened to the micro electrode holder with a set screw (Fig 1B *ss* 2) and it was allowed to slide freely in the hole of the smaller perspex cover (*co* 2) A cannula with an outer diameter of 1 5 mm was fixed to the end of the guide tube for penetration of the dura

The micro-electrode holder was grounded and the micro-electrode connected through a cathode follower to a preamplifier Thalamic and cortical evoked potentials were displayed on an oscilloscope and photographed In some experiments an average of several responses was obtained by feeding the signals to a Nuclear Chicago data retrieval computer Spontaneous cortical activity was recorded on a Grass polygraph either differentially be tween two cortical sites or with ground as a reference

Stimulation

Clicks were made by delivering square 50 μs pulses to an amplifier which fed a loudspeaker The click consisted of various frequencies with the largest peak at 620 Hz A click is a useful stimulus because it produces in the auditory nerve a reasonably well synchronized burst of impulses whose subsequent disper sion through the nervous system can be meas ured in space and time (Rosenzweig & Rosenblith 1953)

The general arrangement for click stimula tion and recording of the sound pressure has been described in detail earlier (Etholm 1975*a*) except that in the present experiments the sound was delivered just outside the outer ear and not through a tube inserted in the outer ear canal

Barbiturates

Sodium pentobarbital was administrated in traperitoneally after satisfactory records had been obtained in the conscious animal During most experiments only one dose was given (30

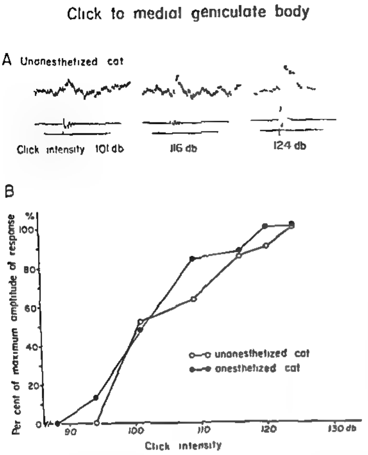


Fig 2 (A) Upper traces show records from the medial geniculate body from an unanaesthetized cat in response to clicks of increasing intensity The second trace gives the microphone record of the click and the lower trace the time calibration—10 ms (B) Input/output curve showing the relation between the size of the MG field potential (*ordinate*) and the click intensity (*abscissa*) without (O) and with anaesthesia (●)

mg/kg), being sufficiently large to give an anaesthetic level where the cat just withdrew the forepaw when pinched

Identification of electrode sites

After a successful track the tissue around the tip of the recording electrode was coagulated by passing 0 2 mA (d c) through the electrode for 20 sec Subsequent histological sections usually revealed easily recognizable lesions During the experiments, control of the electrode sites was attained in the following ways (1) the micro electrode sites was placed according to Horsley Clarke coordinates, (2) the thalamus of the unanaesthetized cat showed a characteristic spontaneous activity, different from that of the overlying structures (Gjerstad & Skrede, unpublished observations), (3) in the medial geniculate body and the auditory cortex typical short latency evoked potentials are produced by the application of a click near the cat's ear (Katsuki et al , 1959, Aitkin et al , 1966, Rose & Galambos, 1952), (4) in most experiments sodium pentobarbital was given intraperitoneally, to compare the activity of thalamic cells before and under anaesthesia Under barbiturate anaesthesia the thalamic complex shows a characteristic spindle activity that has not been recorded from other subcortical structures (Junge & Sveen, 1968) The presence of such rhythmic activity therefore indicated that the electrode was situated in the thalamus

Stages of consciousness

The stages of consciousness were determined according to the behaviour of the cats and their cortical activity A classification using

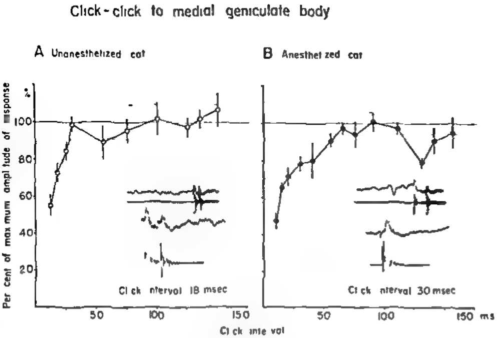


Fig 3 (A) Effect of a conditioning click on a subsequent response to a standard click expressed as percentage of the control response (*ordinate*) *Abscissa* Interval between the conditioning and test click (ms) The two horizontal stippled lines give the range of the control response amplitude The inset shows the MG field response (upper trace) and the two clicks (second trace) The intensified portions of the sweeps are expanded in the two lower traces (B) As (A) but after injection of 30 mg pentobarbital sodium per kg/body weight

five stages was adopted awake/alert, ake/drowsy, light slow wave sleep (LSWS), slow wave sleep (DSWS) and rapid eye ement (REM) sleep (Hess et al , 1953, uvet 1965, Ursin 1968)

RESULTS

Input to medial geniculate body (MG)

In order to investigate the influence of the anaesthesia on the negative field potential wave evoked in the MG input/output curves were made before and after the cat was anaesthetized (Fig 2) The examples at the top (A) show field potentials obtained in the unanaesthetized cat with increasing click intensity from 101 to 124 dB The curves of Fig 2B are constructed from these analogue figures and similar records from the anaesthetized cat The ordinate gives the answer in per cent of the maximum amplitude of the response, and the abscissa is the click intensity The input/output curve had the same steepness in the unanaesthetized as in the anaesthetized cat, the response growing from zero to 100% while the click intensity increased over a domain of 20–30 dB only The absolute value of the click evoked negative wave was roughly similar with or without anaesthesia

Inhibitory processes in MG

The effect of barbiturate on the inhibition were studied in experiments with double clicks Fig 3 A shows the inhibition of the test response in the medial geniculate body of the unanaesthetized cat, and similar data from the same cat after the administration of 30 mg barbiturate/kg body weight (Fig 3B) Examples of the analogue figures are shown as insets in each plot The abscissa represents the click interval and the ordinate the size of the negative field potential evoked by the second click in per cent of the undonditioned response The amplitude of the control responses varied When the cat was unanaesthetized they

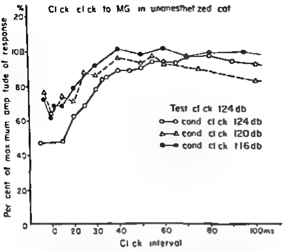


Fig 4 Inhibition of a MG response to a test click of 124 dB produced by previous conditioning clicks of various intensities *Ordinate* Size of the response in per cent of the control response *Abscissa* Interval between the two clicks

ranged from 90 to 110% and when anaesthetized between 95 and 105% In this experiment a single dose of sodium pentobarbital was given after recording the data on which Fig 3 A is based The electrode was left in place in the medial geniculate body in order to record from the same cells after the cat was anaesthetized Before a permanent stage of anaesthesia was reached, however, the cat showed several vigorous muscle contractions In spite of the fixation of the head, it is possible that this shivering damaged the tissue around the tip of the electrode

In the unanaesthetized cat the result showed that the inhibition was about 50% at an interval of 10 ms The response reached the control level at an interval of about 30 ms When the cat was anaesthetized, the inhibition had a longer duration, and the potential did not reach control size before the click interval was 65 ms or more

In the anaesthetized state cyclic inhibition was observed In this example the second inhibitory peak appeared at a click interval of about 130 ms The inhibition observed in barbiturate anaesthesia in these experiments was of a somewhat shorter duration than found earlier (Etholm, 1975*a*), possibly due to

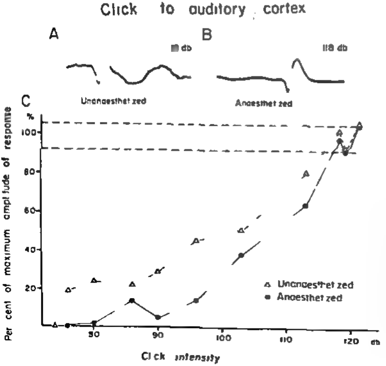


Fig 5 (A) Response from the auditory cortex in a click of 118 dB in the unanaesthetized cat (B) As (A) but after injection of 30 mg pentobarbital sodium per kg/body weight (C) Input/output curves showing the relation between the size of the auditory cortex response and click intensity

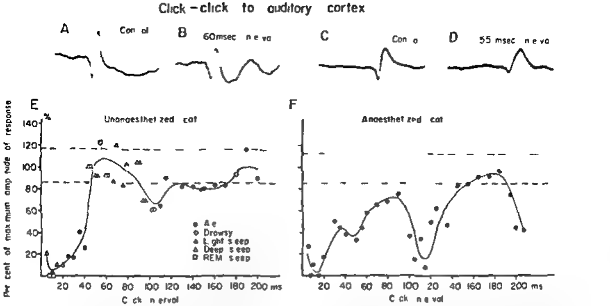


Fig 6 Inhibition of a click-evoked response in the auditory cortex by a preceding click (A) Control response from the auditory cortex to a click of 120 dB (B) Reduction of the control response by a click preceding the test by 60 ms (A D) Sim lar to (A) and (B) but after injection of 30 mg pentobarbital sodium per kg/body weight (E) Graph with the response to the test stimulus plotted against the interval between the conditioning and test clicks The symbols in the insets indicate the different sleep/alert stages in which the animal was when the responses were taken All responses composed by 30 computer averaged responses (F) As (E) but after injection of 30 mg pentobarbital sodium per kg/body weight

a shallower depth of anaesthesia In anaes etized cats increasing strength of the condi ng clicks increased the duration of the ition (Etholm 1975*a*) Similar experi ents were done in unanaesthetized cats (Fig 4) and also here was the inhibition prolonged and augmented

Input to the auditory cortex

To study the output from the MG cells the short latency positive wave of the auditory cortical potential was measured taking this part of the response mainly as an indicator of the effect of the thalamo cortical fibres partly a sign of the afferent volley and partly as an extracellular EPSP Although composed of two processes the deflection gives a measure of the output from the MG cells Input/output curves were made, similar to those in MG by measuring the amplitude of the positive wave Fig 5 A and B are examples of averaged analogue records from a cat before and after anaesthesia respectively Fig 5 C shows the amplitude of click evoked responses obtained by averaging 30 consecutive sweeps in the ca when unanaesthetized and anaesthetized re spectively Each data point of the curve represents one set of averaged response Th ordinate gives the response in per cent c maximum amplitude and the abscissa give the click intensity The answer grew from zer to 100% while the click intensity increase about 40 dB in the unanaesthetized as well a anaesthetized state the threshold in th former condition however being somewha lower Comparing the absolute value of th click evoked response (Fig 5 A and B) th auditory cortical response decreased wit anaesthesia

Duration and size of inhibition in auditory cortex

The inhibition of the click evoked response from the auditory cortex represents the

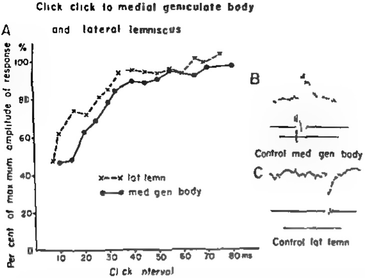


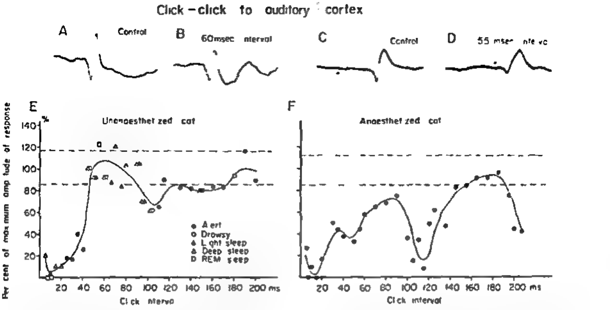
Fig 7 (A) Reduction of responses from the medial geniculate body and from the lateral lemniscus to a click of 121 dB caused by a conditioning click of 121 dB delivered at various intervals before the test click The responses are expressed in per cent of the control (B) Response from the medial geniculate body (C) The killed end response from the lateral lemniscus taken after the medial geniculate body had been destroyed The second trace in each assemblage gives the microphone record of the click and the lower trace the time calibration 10 ms

cumulative inhibition from the organ of Corti up to and including the MG and the auditory cortical cells Fig 6 shows the results obtained from such an experiment in a cat before and after administration of sodium pentobarbital In Fig 6A–D each example represents 30 averaged analogue signals Fig 6E shows the results obtained when the cat was unanaesthetized and Fig 6F when anaesthetized The ordinate gives the answer in per cent of the amplitude of the unconditioned response and the abscissa the click interval

When unanaesthetized the cat's stage of arousal or sleep was determined using the classification described in methods

Compared with Figs 3 and 4 it is obvious that the inhibition was stronger when the click had passed the relay cells in the medial geniculate body The test reponse was zero when the click interval was about 10 ms and the response did not return normal until the click interval was increased to about 40 ms In these experiments there was a second period of inhibition when the click interval was about 100 ms The cyclic inhibition was observed both when the cat was awake/drowsy or showed the characteristics of light slow wave sleep deep slow wave sleep or REM sleep

Fig 6F shows the result obtained from the same electrode site in the same cat but during light pentobarbital anaesthesia The inhibition increased in strength as well as duration when the cat was anaesthetized In this example the test potential did not reach the control size until the click interval was about 150 ms The tendency to cyclic inhibition at about 100 ms was more pronounced when the cat was anaesthetized A new period of inhibitory process was observed with a click interval around 190–210 ms Correspondingly, repetitive evoked potentials in response to a single click was often observed, particularly when the cat was drowsy Such reverberations could however not be obtained in the state of alertness, for example when the cat was excited by the sight of milk The regularity and amplitudes of the cyclic discharges increased with anaesthesia



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Duration and size of inhibition in auditory cortex

The inhibition of the click-evoked response from the auditory cortex represents the

DISCUSSION

The input/output curve of the responses recorded from the MG in the unanaesthetized cat had the same steepness as that found during subsequent moderate anaesthesia (Fig 3) indicating that the high fidelity of transmission was retained. The absolute value of the click evoked response in the MG was hardly depressed by the barbiturate anaesthesia. In the auditory cortex, however, the absolute value of the evoked response showed a little reduction during barbiturate anaesthesia. This may indicate that cortical cells are more susceptible to barbiturate than thalamic relay cells.

The MG was found to contribute most to the inhibition in the upper auditory pathway (Fig 8). Measured as equivalent reduction of sound pressure in dB the total inhibition caused by a strong preceding click was about 13 dB when recorded in the lateral lemniscus. When recorded in the auditory cortex the inhibition represented a reduction of the input click of about 34 dB. The difference in inhibition recorded from the two locations can be interpreted as due to the combined effect of pre- and postsynaptic inhibition added at the MG level.

These data obtained in the unanaesthetized cat correspond to the findings in the barbiturate anaesthetized animal (Aitkin et al., 1966, Kitahata et al., 1969, Etholm, 1975*a*). It correlates with the findings from the somatosensory and visual system in which thalamus has been found to be the seat of strong inhibition (Poggio & Mountcastle, 1963, Andersen et al 1964, Burke & Sefton, 1966). After anaesthesia the MG inhibition was prolonged. The duration of the inhibition (the time before the response reached the control value) was increased by over 100% when recorded postsynaptically in MG and even more when estimated from the size of the response of the auditory cortex.

The prolongation of the inhibition during barbiturate anaesthesia is in accordance with the experimental data of Poggio & Mountcastle (1963) from VPL and of Aitkin et al (1966) from MG. Even more striking effects have been reported by Bloedel & Roberts (1969) who found a change from facilitation to inhibition of cerebellar Purkinje cells on addition of barbiturate. Although this prolongation of inhibition or even facilitation/inhibition reversal most likely is a main factor in controlling neuronal activity during anaesthesia, the basic mechanisms for the nervous effect of barbiturate are, however, still unknown. Aitkin et al (1966) suggested that the influence from the reticular formation had a depressive effect on the inhibitory interneurones in the unanaesthetized cat. During anaesthesia this effect was reduced and hence followed by an increase of the click-evoked inhibition. An even more general explanation is suggested by Løyning et al (1964), finding that barbiturate acts mainly on the afferent terminals resulting in less transmitter release and reduced synaptic potentials.

Another explanation derives from experiments which have shown that barbiturate exerts a local action when administrated directly to the neurones by iontophoretic technique (Krnjević, 1965, Bradley & Wolstencroft, 1965). Another possibility is the hypothesis that barbiturates exert a direct inhibitory action on an enzyme (Eccles, 1965) destroying the transmitter released from the synapses responsible for presynaptic inhibition. Our finding that the inhibition is more pronounced when the neuronal volley had passed the MG relay cells, does not allow us to choose between these hypotheses, but indicates that sodium pentobarbital somehow changes the properties of the inhibitory systems operating on the MG cells.

Although the number of experimental animals was restricted we conclude that the same inhibitory function found in MG of anaesthetized animals probably is present in the unanaesthetized state, but to a lower degree. The tendency to cyclic inhibition found in VPL by Andersen et al (1964) and in MG by

Etholm (1975*a*) and in VL of unanaesthetized animals was also found in our experiment The cyclic discharges sometimes recorded in the auditory cortex after a single click probably have the same mechanism as this recurrent inhibition shown in the thalamus The similarity between the cyclic discharges recorded during drowsiness and during anaesthesia further supports the idea that the same inhibitory mechanism is present also in the unanaesthetized cat

ZUSAMMENFASSUNG

Eine Methode die das wiederholte Registrieren von subcorticalen Strukturen mit Mikroelektroden erlaubt ohne irgendeine Art von Medizin zu benutzen ist beschrieben worden

Diese Methode wurde in Verbindung mit der konventionellen Implantationstechnik angewendet um durch einen Klicks hervorgerufene Potentiale und die inhibitorischen Mechanismen im auditorischen System bei Katzen zu untersuchen Im Corpus geniculatum mediale wurden die durch einen Klicks hervorgerufenen Potentiale kaum von moderaten Dosen von Barbituraten beeinflusst und in der auditorischen Cortex nur in geringem Masse Beim nicht anasthesierten Tier wurde der bedeutendste Beitrag zu der durch einen Klicks hervorgerufenen Inhibition im Gehorsystem durch Mechanismen im Corpus geniculatum mediale verursacht Beim nicht anasthesierten Tier war die Inhibition sowohl in ihrer Grosse als in ihrer Dauer geringer als beim anasthesierten Tier Die Zellen im Corpus geniculatum mediale zeigten eine Tendenz zur zyklischen Inhibition bei der nicht anasthesierten Katze jedoch nicht so regelmassig wie wenn die Katze ein Barbiturat bekam Verschiedene Theorien daruber wie Barbiturate auf das Gehórsystem wirken konnen wurden besprochen

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ADVANCED MENIERE S DISEASE

A Study of 356 Severely Disabled Patients

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(Received May 28 1975)

Abstract : A selected material of 356 patients with Meniere s disease all severely disabled by paroxysmal vertigo was analysed The question under study was to what extent and in what way the severe vertigo is reflected in the caloric reaction and in the level of hearing The caloric response expressed as differences in right/left sensitivity was reduced in 59% and exaggerated in 1% of the patients in the remaining 40% it was normal The median values for pure tone audiogram speech reception threshold and speech discrimination score were 56 dB 60 dB and 54% respectively The hearing deteriorated considerably in the early stage of the disease but the hearing level stabilised with time A parallelism between acoustic and vestibular dysfunction was demonstrated Reduction of the pure tone threshold was accompanied by a progressive decrease in caloric response The frequency of bilateral cases increased with lengthening duration of illness All patients included in this investigation have since undergone operation

Large variations in the course degree of severity and inner ear dysfunction are characteristic of Meniere s disease This multifacetted disease pattern makes evaluations of the long term prognosis very uncertain One way of increasing our knowledge of the nature and development of the disease is to study a strictly selected well defined group of patients This was done in the present investigation

The patients included were all severely incapacitated by vertigo and were considered in need of surgical therapy It is probably no exaggeration to describe this series as representing the most severe and most disabled Meniere patients to be encountered in this country

Several problems were attacked, viz what are the levels of vestibular and acoustic function in these patients with severe vertigo? Is the severe vertigo reflected in a clearly depressed caloric response? What is the influence of the duration of the disease on the function of the inner ear?

The present investigation is to some extent a continuation of previous studies (Enander & Stahle 1967 Stahle & Bergman 1967 Enander & Stahle 1969) The material was further selected however and had a considerably more uniform profile

PATIENTS

The material comprised 356 patients with Meniere s disease selected during a 12 year period from December 1961 to December 1973 All of them had been admitted to the Department of Otorhinolaryngology of the University Hospital Uppsala for investigation

Common to all of them were (*a*) that they suffered from severely disabling attacks of vertigo (*b*) that these attacks were refractory to various forms of medical therapy, (c) that in bilateral cases the labyrinth from whic the

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Table I *General profile of the case material*

Total number of Meniere patients		356
Unilateral		322
Right side	164	
Left side	158	
Bilateral		34
Male		206
Female		150
Mean age		50 years (range 23–77 years)
Mean duration of illness		[illegible] years (range 2 months – 38 years)

vertiginous attacks were induced could be clearly defined

All 356 patients underwent operation subsequent to the investigations described below The method of operation was simple mastoidectomy combined with ultrasonic irradiation of the vestibular part of the inner ear (Sjoberg et al , 1963 Stahle 1975) The results of these operations will be reported in this journal

METHODS

Pure tone audiometry was performed on all 356 patients The mean value for the frequencies 500 1000 2000 and 3000 Hz was calculated *The speech discrimination score* and *speech reception threshold* were measured in 234 patients The reason that the two latter examinations were not performed on all patients was that up to 1968 we did not have access to complete audiological services After that date all three hearing tests were included routinely in the investigations *Caloric tests* were carried out on all patients In 4 patients the reaction could not be evaluated with certainty (3 with a perforated tympanic membrane and 1 with fixation nystagmus) and the final report thus comprised 352 patients

For the caloric test the patient lay supine with the head raised 30° The water temperatures were 30° and 44°C and the duration of syringing 30 sec with a 5 minute interval between each syringing Electronystagmography with closed eyes was invariably used (Aschan et al , 1956) The reactions were evaluated on the basis of the *maximum intensity* (Stahle, 1958, 1968) By this is meant the mean eye speed in the slow nystagmus phase during a 10 second period at the peak of the caloric reaction The percentage difference between the right and left ear was assessed according to Jongkees & Philipszoon (1964) Only differences exceeding 20% (equivalent to about 2 5× the standard deviation) were regarded as clearly pathological We are aware of the liberality of this figure, but we selected it partly to simplify the calculations and partly to avoid the risk of overdiagnosis (Stahle & Bergman, 1967) The 34 bilateral cases were also evaluated separately by another method each ear being assessed separately in comparison with existing normal values

The data were treated in a Siemens 305 Process Computer

General profile of the case material

The mean age of the patients was 50 years (range 23–77 years) and the mean duration of

Table II *Hearing and caloric response in relation to duration of illness*

	Duration of illness (years)					
	0–2	3–5	6–8	9–13	14–	Total
Pure tone audiogram (dB)						
Median	56	56	55	55	56	56
Mean	55	54	55	56	56	55
No of patients	73	100	64	60	59	356
Speech discrimination score (%)						
Median	49	56	58	52	39	54
Mean	52	51	54	52	46	51
No of patients	54	65	50	31	34	234
Speech reception threshold (dB)						
Median	61	58	57	56	67	60
Mean	62	59	59	61	63	60
No of patients	54	65	50	31	34	234
Caloric response (difference in max intensity in right/left sensitivity) (%)						
Median	23	26	28	30	25	27
Mean	25	25	28	36	25	27
No of patients	66	94	61	51	46	318

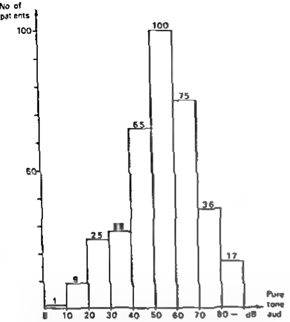

Fig 1 Histogram illustrating the results of pure tone audiograms from 356 patients with advanced Meniere's disease Only 4 patients were completely deaf The median is 56 dB

illness 8 years (range 2 months–38 years) Only one patient had a shorter duration of illness than one year The sex distribution and location of the disease are shown in Table I The overrepresentation of men has been pointed out previously (Cawthorne & Hewlett, 1954, Stahle & Bergman, 1967, Harrison & Naftalin 1968, Hedgecock, 1968)

Results of hearing tests and caloric tests

These results are presented in Table II in relation to the duration of the disease In the calculations of mean values four completely deaf ears were given a hearing loss value of 100 dB

For pure tone audiogram the median value was 56 dB and the mean 55 dB (Fig 1) For speech discrimination score the median value was 54% and for speech reception threshold 60 dB (Fig 2) The number of observations for these two latter parameters was only 234, for the reasons given above

The differences in maximum intensity presented in Table II represent the caloric test results from 318 unilateral cases The 34 bilateral cases were estimated separately and the results are presented in Table V

As seen in Table II, the hearing level, expressed by the pure tone audiogram, remained fairly constant after the initial loss during the first 2 years of illness For speech discrimination, on the other hand, a change in the median value from 49% to 39% was noted with lengthening of the duration of illness This reduction can be regarded as an expression of continuing degeneration in the inner ear (cf Table IV)

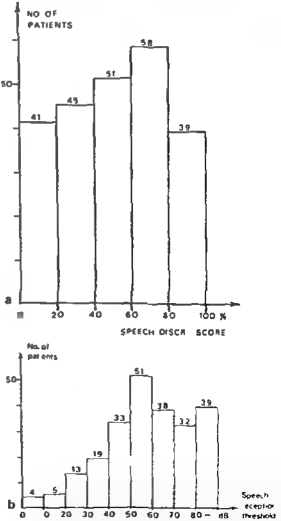

Fig 2 Histogram presenting (*a*) speech discrimination score and (*b*) speech reception threshold in 234 patients The medians are 54% and 60 dB

Table III *Duration of illness and incidence of bilaterality*

	Duration of illness (years)					
	0–2	3–5	6–8	9–13	14–	Total
Total no of patients	73	100	64	60	59	356
No of bilateral	[illegible]	6	3	7	12	34
Rate (%)	8	6	5	12	20	10

The recordings of differences in maximum intensity of caloric response indicate that the vestibular hypofunction in the diseased ears progresses, to reach a maximum after 9 to 13 years (Table II) From that period both the median and the mean value for the differences between the two labyrinths decreased One possible explanation for this phenomenon is that the disease may have become bilateral, without this being clearly manifested as bilat eral hearing loss or in any other way

Duration of illness and bilaterality

As reported previously the number of bilateral Meniere cases increases with the duration of the disease (Stahle & Bergman, 1967) Among the 356 patients of the present investigation 34 were bilateral It can be seen in Table III that the frequency of bilateral cases increased with time—from 8% to 20% in 14 years

Age and speech discrimination

Younger patients had a better speech dis crimination score than older ones (Table IV) In a χ^2 test the differences were statistically significant ($p<0.01$) No significant deterioration of the hearing loss expressed by the pure tone audiogram and speech reception thresh old in relation to age was found

Comparison between results of different hearing tests

Fig 3 illustrates the hearing loss in 234 pa tients, as expressed by pure tone audiograms and speech discrimination scores A distinct parallelism between these two parameters can be seen This is evident both by the fact that

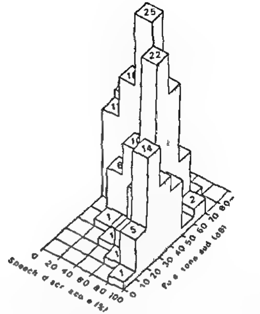

Fig 3 Comparison between speech discrimination score and pure tone audiogram in 234 patients with advanced Meniere s disease The connection is evident ($p<0.001$)

the majority of observations are grouped on the diagonal of a coordinate system, and by the empty squares in one of the corners, which implies a lack of cases with comparatively poor discrimination score in relation to the pure tone loss

The caloric response

Among the 318 unilateral cases, the caloric response was reduced in 188 (59%), ex aggerated in 4 (1%) and normal in 126 (40%) It should be noted, however, that normal means no significant difference between the responses from the right and left ear Phenomena such as directional preponderance were not taken into consideration in this investiga tion

Table IV *Speech discrimination scores (%) in relation to age in 234 patients with severe Meniere s disease*

	Per cent				
Age (years)	0–20	21–40	41–60	61–80	81–100
–39	4	4	16	17	14
40–49	8	15	15	16	15
50–59	[illegible]	15	14	18	6
60–	11	11	6	7	4

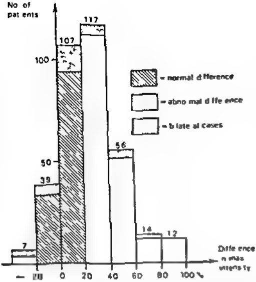

Fig 4 The caloric response of 352 Meniere patients disabled from severe paroxysmal vertigo Maximum intensity is an expression of the eye speed of the slow nystagmus phase at the peak of the caloric response Differences in right/left sensitivity of up to 20% have been regarded as normal The disease was bilateral in 34 patients Abnormal difference i e reduced caloric response was recorded in 199 patients

The results of the caloric test in all 352 patients are illustrated in Fig 4 An abnormal difference where the affected ear (the ear with the greater hearing loss in bilateral cases) had a weaker reaction than the other ear was found in 199 patients and an abnormal difference where the affected ear had the stronger reaction was found in 7 patients (4 unilateral and 3 bilateral) In the remaining 146 patients no significant difference in right/left sensitivity was noted according to the criterion for abnormality given above under Methods

The finding of patients with a stronger caloric response from the diseased labyrinth is consistent with previous observations (Stahle & Bergman 1967, Thomas & Harrison, 1971)

In the 34 bilateral cases the caloric excitability was estimated separately (Table V) in relation to data from normal subjects (Stahle, 1956 1958) Caloric hypofunction was more frequently found in the poorer hearing ears than in the better ears Hyperfunction was recorded in the better hearing ear in one patient

Table V *Caloric reaction in 34 patients with bilateral Meniere s disease*

	Poorer hearing ear	Better hearing ear
Normal	23	28
Reduced	11	5
Increased	–	1

Normal range for cold plus hot water reactions 10–53°/sec (2×S D)

Correlation between caloric response and hearing loss

According to a previous study there is a correlation between caloric hypofunction and hearing loss in Meniere's disease (Enander & Stahle, 1969) The question arose if this correlation was distinct in the present, more selected, material of patients with severe vertigo

As mentioned above, normal right/left sensitivity was recorded in 40% of the 318 *unilateral cases This means that in 126 pa*tients severely incapacitated by paroxysmal vertigo and considerable hearing impairment, the caloric test results were not pathological For the material as a whole, however (Fig 5), a significant correlation was found between the degree of caloric impairment and the degree of hearing loss ($c=0$ 90)

DISCUSSION

As is evident from the above, both hearing loss and reduction of caloric excitability increase with the duration of symptoms It has been found, further, that the hearing deteriorates considerably within a short period of time during the early stage of the disease Even in the group of patients who had had their disease for less than 3 years the mean values for pure tone audiogram SRT and speech discrimination score lay at 55 dB, 62 dB and 52% respectively These values then showed no appreciable change with time, except for some further reduction of the speech discrimination capacity in patients with a very long duration of illness

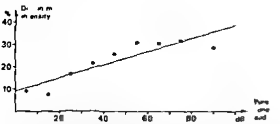

Fig 5 Comparison between caloric response (diff in max intensity) and pure tone audiogram in 318 cases with advanced unilateral Meniere's disease Increase in hearing deterioration is accompanied by further depression of the caloric response ($p<0.001$)

The observation that the dominant part of the hearing loss develops relatively early in the course of the disease has been made previously (Enander & Stahle 1967) In that study in a large group of patients with a total duration of illness of up to 6 months the average hearing loss (pure tone audiogram) was 35 dB while in the next duration group (6–12 months) it had progressed to 50 dB The report by Thomas & Harrison (1971) that the deterioration in hearing is far greater in the first 5 year period than in the subsequent years is completely in line with our observations

The braking of the functional reduction at a hearing threshold of 50–60 dB and a speech discrimination capacity of 50–60% seems to be a common phenomenon in Meniere's disease A similar tendency is seen in Hedgecock's material (1968) Patients with more advanced hearing loss are few and in our experience total deafness is uncommon The early onset of considerable hearing loss seems to motivate special therapeutic attention in the initial stage of the disease particularly in patients incapacitated by severe vertigo

As regards the caloric response in the present series it behaved similarly to the hearing loss A reduction was observed even during the first 2 years of the disease The mean values then rose slightly in the following 11 years In patients with an even longer duration of illness the difference in right/left sensitivity decreased somewhat which might be interpreted uncritically as a sign of recovery of the labyrinth This is probably not the case however The explanation may be that during the protracted illness the apparently healthy or better ear has been afflicted by vestibular impairment even though there is no characteristic hearing loss This should reduce the discrepancy in caloric response between the two labyrinths This interpretation would seem reasonable in view of the remarkably high figures for bilaterality reported in recent years Jongkees (1971) denoted two thirds of the Meniere patients attending his clinic in Amsterdam as bilateral Thomas & Harrison (1971) reported 32% bilateral cases in their English series of 564 patients We ourselves have shown previously that the frequency of patients with bilateral involvement increases with the duration of the disease (Bergman & Stahle 1967) an observation which was also made in the present study It is also known that the disease often has its onset mono symptomatically i e a vestibular dysfunction may occur without any signs of cochlear involvement (Enander & Stahle 1967 Thomas & Harrison 1971) It is thus fully conceivable that bilaterality of the vestibular dysfunction alone underlies the diminishing right/left difference noted in our study

In occasional cases there was a stronger caloric response from the affected ear than from the apparently intact ear In 7 patients including 4 with bilateral symptoms the caloric response was definitely stronger on the affected side (Fig 4) This phenomenon has been described previously (Stahle & Bergman 1967 Thomas & Harrison 1971) The explanation might be that the semicircular canals in the better hearing ear are even more diseased than in the ear with the greater hearing loss

As severe paroxysmal vertigo was a criterion for inclusion in the present material it may seem remarkable that reduced caloric response was recorded in only 59% of the patients This corresponds fully with our previous findings in a less highly selected series (Stahle & Bergman 1967) In other investigations on the other hand considerably higher figures have been reported Cawthorne &

Hewlett (1954) found "canal paresis" in 83% and Thomas & Harrison (1971) in 80%. The discrepancies in the frequency of cases with reduced caloric response may be due to different opinions as to what is normal. We have placed the limits for a normal difference in right/left sensitivity at as wide as 2.5 S.D., for the reasons given above, whereas the cited authors have used limits of 2 S.D. In view of the dominance of vertigo in our series of patients it must be concluded that disabling paroxysmal vertigo is not reflected in marked caloric depression either in the frequency of cases or in the degree of depression. This result is not surprising as the caloric response is mainly an expression of the functions of the semicircular canals and leaves the question of the state of the utricle and saccule unanswered. The occurrence of a permanent dysfunction in the utricle is contradicted, however, by reports on normal counter rolling in patients with unilateral Meniere's disease (Terins 1970).

ZUSAMMENFASSUNG

Ein ausgewähltes Material von 356 Patienten mit Mb. Meniere, die alle durch anfallartigen Schwindel schwer invalidisiert waren, wurde analysiert. Die Fragestellung der Untersuchung war, in welchem Ausmass und auf welche Weise der schwere Schwindel mit der kalorischen Erregbarkeit und dem Hörvermögen in Zusammenhang steht. Die kalorische Erregbarkeit, die als Empfindlichkeitsunterschied zwischen rechts und links ausgedrückt wird, war in 59% der Fälle vermindert und bei 1% der Patienten erhöht, die restlichen 40% waren normal. Die Durchschnittswerte für Reintonaudiometrie, Sprachhörschwelle und Sprachdiskriminationsleistung waren der Reihe nach 56 dB, 60 dB und 54%. Das Hörvermögen verschlechterte sich im frühen Stadium der Erkrankung beträchtlich, jedoch stabilisierte sich die Hörleistung mit der Zeit. Eine Parallele zwischen akustischer und vestibularer Funktionsstörung wird nachgewiesen. Eine Verminderung der Reintonhörschwelle war von einer fortschreitenden Abnahme der kalorischen Erregbarkeit begleitet. Die Häufigkeit von beidseitiger Störung wächst mit der Dauer der Erkrankung. Alle Patienten, die diese Untersuchung umfasst, haben sich später einer Operation unterzogen.

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ULTRASOUND TREATMENT OF MENIERE'S DISEASE

Long term Follow-up of 356 Advanced Cases

J Stahle

From the Department of Otolaryngology, University Hospital Uppsala Sweden

(Received May 28, 1975)

Abstract Twelve years experience of ultrasound treatment of 356 patients is presented For evaluating the results a new multifacetted scoring system was developed where each individual patient was evaluated on the basis of four parameters According to this evaluation 71% of the patients improved after the operation while 29% remained unchanged or deteriorated Because of lack of improvement 42 patients underwent reoperation by different methods in half of them ultrasonic irradiation was again used The hearing deteriorated in about 40% of the patients to which result the long observation times also contributed The caloric response became reduced in 70% of the patients Marked depression of the caloric response characterized the patients who were relieved of vertigo Transient facial paralysis in 4 patients was the only omplication

As long as the pathogenesis of Meniere's disease remains uncertain, medical as well as surgical treatment has to be symptomatic In most patients the condition can nowadays be satisfactorily controlled by different kinds of conservative therapy Long term treatment with diuretics has been found effective, particularly against vertigo (Klockhoff et al, 1974) Surgery has to be considered in the very small number of patients who in spite of longstanding medical therapy are still disabled by frequent and severe attacks of vertigo When choosing the form of surgical therapy it has to be taken into account that the disease may afflict both labyrinths in about a quarter of all cases—perhaps even more Operations in which the remaining hearing is consciously destroyed should therefore be avoided as far as possible

The whole art of the treatment of Meniere's disease with ultrasound rests upon the necessity of applying an optimum dose of energy to *the vestibular end organs so that destruction* or near destruction can be achieved but without causing further damage to the organ of Corti or the facial nerve It is the attainment of perfect balance between these two effects that is the key to successful treatment

The ultrasound method has been used at the ENT clinic in Uppsala since November 1961 During the 12-year period ending in November 1973, 356 patients have been treated in this way Our principles in selecting the patients, and several characteristics of this group have been described in a previous article (Stahle, 1975)

In the present paper the long term results of the operation, as judged from questionnaires and otoneurological examinations will be presented

Technique of operation

The original apparatus designated Ultrapoint', which emits focused 1 25 MHz ultrasound, was used (Johnson, 1967) Up to 1965 the gracile transducer was supplied with interchangeable steel tips Since that time we have gone over to cone shaped teflon tips with an end diameter of 2 mm

This work was supported by the Swedish Medical Research Council (Project No B74 17X 3908-02 and B75 17X 3908-03A)

The semicircular canals were exposed through a simple mastoidectomy which was performed under local anaesthesia The otic capsule was thinned out with a diamond drill in the target area When we first started these operations we directed the ultrasonic beam towards the ampullar part of the lateral semicircular canal, but since 1963 we have directed it towards the thinned out capsule in the junction between the lateral and superior canals, which region lies more distant from the facial nerve and the cochlea With this approach the ultrasonic energy will affect mainly the superior and lateral ampullae and the vestibule During the last two years if the anatomy has allowed, the posterior semicircular canal has also been irradiated, with the aim of improving the result further The technique of operation has been described in detail previously (Sjoberg et al, 1963, Stahle, 1975)

Only two surgeons have performed the actual irradiation—A Sjoberg, M D, Professor and previous head of the ENT department in Uppsala, and the author

Composition of patient material and observation times

The investigation was carried out by two approaches—partly by means of a questionnaire sent to the patients (Fig 1), and partly by a retrospective analysis of the results of otoneurological examinations at the hospital At least one year should have elapsed between the operation and the time of the investigation, for inclusion of a patient in the series

Concerning the questionnaire, 356 patients fulfilled the criterion of a 1-year observation time Thirteen of these patients could not be reached because of a change of address The remaining 343 patients received the questionnaire, and 303 of them answered Of the 40 for whom no replies were received, 14 had died, *thus leaving 26 who declined to answer* Of the 303 patients who answered the questionnaire 279, who had undergone ultrasound treatment either once or twice, were included in the

(a) Since your operation is your *dizziness* ☐ completely absent ☐ *less pronounced* ☐ unchanged

(b) Since your operation is your *hearing* ☐ unchanged ☐ improved ☐ worse

(c) Since your operation are the *noises in your ears* ☐ unchanged ☐ completely absent or decreased ☐ more disturbing than before

(d) Since your operation is your *ability to work* ☐ unchanged ☐ increased ☐ lessened

Fig 1 The questionnaire sent to 356 patients with Meniere's disease treated with ultrasound The observation time was at least one year

investigation The answers from the remaining 24 patients, all of whom had undergone reoperation by another method than ultrasonic irradiation, were not included in this part of the study as it was apparent that in their reply the patients could not differentiate with certainty between the results of the two operations, these patients are reported in connection with the multifacetted scoring system

For the 279 patients who were included, the observation times were 1–6 years in 82 cases and 7 years or more in the remaining 197

Otoneurological examination was performed one year or longer after the operation in 268 patients (Table I) The results of 110 operations in patients with a shorter

Table I *Follow up times in otoneurological examinations*

Follow up time (years)	No of ultrasound operations	
<1		110
1–2	124	
3–4	79	
5–6	40	
>7	25	268
Total	268	378
No of patients operated on		
once		356
twice		2
Total no of ultrasound operations		

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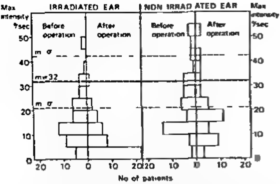

Fig 3 Caloric response in patients with bilateral Meniere's disease 47 patients were tested before the operation and 43 of these satisfied the requirement of an observation time of at least one year Caloric response has been expressed as maximum intensity and the values for the cold and warm water reactions have been summed The mean value for normal individuals is 32°/sec with S D 10 The majority of patients had a weak response both on the irradiated and on the non irradiated side

compared with the preoperative value was recorded in 70% Even an increased reaction was noted in 12% The 236 patients reported in Fig 2 also include some bilateral cases where the caloric reaction was absent on the poorer side

The response to caloric stimulation in all bilateral cases before and after the operation is illustrated in Fig 3 The reason that the results are reported separately is that the method of Jongkees & Philipszoon (1964) is not suitable in bilateral cases Each labyrinth should be assessed separately in relation to data from normal individuals (Stahle, 1975) On the irradiated side, in many cases the caloric response was depressed both before and, especially after the operation On the non irradiated side more values were within normal limits Generally, however there was a trend towards abnormally weak reactions even on this side

Multifaceted scoring system

An attempt at a more penetrating and, in particular a more individual evaluation of the operation results is presented in Fig 4 This analysis included 229 patients, all of whom satisfied two requi

the questionnaire, and (*b*) they had undergone follow-up examination one year or longer after the operation The effect of the operation on the vertigo, ability to work, hearing and tinnitus in each individual case was assessed and given a score of −1, 0, 1, 2, 3 or 4 The best result was thus ascribed 4 points, which a patient received if, at the same time, the vertigo was completely absent, the ability to work was increased and the hearing and tinnitus had not deteriorated The poorest result was −1 The hearing was assessed with pure tone audiometry Failures who later underwent reoperation by another method than ultrasound were classified as unchanged with respect to vertigo, tinnitus and ability to work, while their hearing was evaluated from available audiograms

Improvement of varying degrees from 1 to 4 points was noted for 71% of the patients, while 14% were considered to be unchanged and 15% deteriorated (Fig 5) When the scoring evaluation was based on the speech discrimination score instead of the pure tone audiogram, it was found that of 144 patients, 65% were improved, 16% unchanged and 19% worse

Eight patients stated in the questionnaire

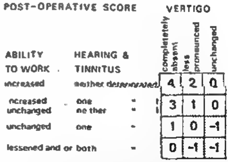

Fig 4 Scheme for individual scoring of the results of ultrasonic treatment in Meniere's disease The best results have been given 4 points the poorest −1 Results between 1 and 4 have been considered to represent various degrees of improvement The following is one example of the scoring A patient who reported subjectively less pronounced vertigo increased ability to work and unchanged tinnitus and whose pure tone audiogram showed un- ... red with the preoperative, was

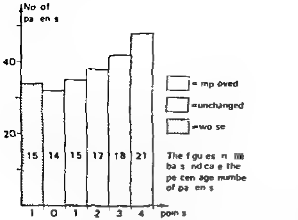

Fig 5 The results of ultrasonic treatment in 229 patients with Meniere's disease based on the new multifacetted scoring system presented in Fig 5 The observation time was at least one year in all cases

that they had become free from vertigo but that their ability to work was lessened An explanation for this unexpected result was obtained in seven cases the underlying factors being difficulty in maintaining balance mental depression severe tinnitus and headache

Complications

In four patients transient facial paralysis occurred during or immediately after irradiation (all in the early stages of the treatment series In all cases the paralysis regressed spontaneously within one to three months No other complications were seen

DISCUSSION

In the questionnaire 40% of the patients stated that their hearing was worse after the operation This figure is in good agreement with the results of different hearing tests which have shown hearing deterioration in 42–49% of the patients This loss can be attributed in part to a direct ultrasonic effect on sensory neural elements in the cochlea which will have occurred despite our efforts to avoid direct irradiation of the cochlea Supporting this assumption are results in experimental animals which have shown damage to the cochlea following direct irradiation (Crysdale & Stahle 1972 Stahle & Sugar 1973) On the other hand in recent investigations the cochlea in guinea pigs has remained intact both functionally and morphologically after irradiation of the semicircular canals (Lundqvist et al 1975)

The long observation times in this series of patients give rise to the question however to what extent the natural course of the disease may have contributed to the hearing loss A good idea of the importance of the time factor can be obtained by comparing the hearing course postoperatively in 43 bilateral cases (Fig 6) Hearing loss (deterioration of at least 10 dB of the pure tone threshold) was noted in 53% of the patients on the operated side and in 30% on the non operated side This may be regarded as evidence that the disease in combination with time was partly the cause of the postoperative deterioration in hearing

When the results were compared with our previous findings which partly concerned the same patients (Sjoberg & Stahle 1965 Drettner et al 1970) good agreement was found In 1970 we reported deteriorated hearing (pure tone audiogram) in 36% of the patients while the hearing in the remainder was unchanged or improved This may be compared with the figure of 42% in the present series The small discrepancy can probably be explained by longer observation times in the later series as well as the fact that a frequency of 3 000 Hz has been added to the three frequencies used previously in the hearing assessment

The speech discrimination score postoperatively was deteriorated in 49% of the patients unchanged in 34% and improved in 17% (Fig 2) In view of previous reports on deterioration of the discriminatory ability of Meniere patients with age (Stahle 1975) it would seem that postoperative speech audiometry does not reveal cochlear damage to any higher extent than tone audiometry

Ultrasonic irradiation leads to depression of the function of the semicircular canals as has been previously shown in both caloric and rotatory tests (Stahle & Sahl 1964) This fact is now further corroborated by the present

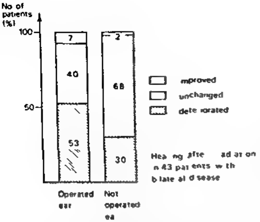

Fig 6 The hearing after ultrasonic treatment of one ear in 43 patients with bilateral Meniere's disease expressed in pure tone audiograms The observation times are at least one year The hearing deteriorated in the non operated ear in 30% of the patients and in the operated ear in 53%

findings, of an absent or decreased caloric reaction postoperatively in 70%, no change in 18% and an increase in 12% The frequency of cases with a depressed response has been found to be somewhat higher now than 5 years ago (cf Drettner et al, 1970)

In order to study more closely the therapeutic importance of postoperative depression of the caloric response, we have compared this with the symptom vertigo (Table II) This analysis comprised 200 patients with unilateral disease, of these, 166 had undergone operation once and 34 had been reoperated on later In these 34 patients we considered the vertigo to be unchanged The observation time was at least one year It is concluded that patients in whom the irradiation has led to absence of a caloric reaction have the greatest chance of becoming completely free from vertigo A χ^2 test revealed a dependence ($p<0.05$)

The question must then be asked how is the postoperative hearing in patients with no caloric reaction? It is often deteriorated (Table III) χ^2 testing revealed a statistically significant dependence ($p<0.05$) As preservation of the hearing is one of the basic principles in the ultrasound method, the surgeon now finds himself in a dilemma An increase of the energy dose may increase the

Table II *A comparison between caloric response and vertigo in 200 patients with unilateral Meniere's disease treated with ultrasound*

Caloric reaction	Vertigo: Completely absent	Less pronounced	Unchanged
Absent	43	21	5
Reduced	29	24	16
Unchanged	14	12	11
Increased	III	9	6

freedom from vertigo, but at the same time will give a greater risk of hearing deterioration

Finally, for comparison some results of ultrasound treatment from other hospitals may be mentioned James (1970) has reported that 85% of his patients became free from vertigo, but in several cases not until after repeated irradiations Deteriorated hearing was found in 44% Sørensen (1970), who uses the same method and has the same equipment as we in Uppsala, has stated that 72% of his patients had complete cessation or a considerable decrease of vertigo Dionne et al (1972) have reported satisfactory control of dizziness in 75% while hearing was completely lost in 20%, significantly decreased in 40% and preserved in 40% Basek (1973) reviewed reports of the treatment of 560 patients operated on with a lateral canal or round window approach, 20% were described as failures

Most of the reports hitherto have been made in the conventional way, where vertigo, hear-

Table III *A comparison between caloric response and hearing in 236 patients with Meniere's disease treated with ultrasound*

Caloric reaction	Pure tone audiogram: Improved	Unchanged	Decreased
Absent	7	36	48
Reduced	7	37	30
Unchanged	[illegible]	24	13
Increased	8	12	

ing and tinnitus have been reported separately In our opinion this gives an incomplete description of the individual course For this reason we have suggested evaluation utilizing a scoring system, which also includes the ability to work This latter has been motivated by our confirmed view that the rehabilitation provided by an improved ability to work has been experienced by the patients as a very important benefit, even if their vertigo has not been completely eliminated

ZUSAMMENFASSUNG

Es wird uber eine 12jahrige Erfahrung mit Ultraschallbehandlung von insgesamt 356 Patienten berichtet Zur Auswertung der Ergebnisse wurde ein neues Vielfacetten Punktesystem entwickelt wo jeder einzelne Patient an Hand von vier Parametern bewertet wird In Ubereinstimmung mit dieser Auswertung hatten sich 71% der Patienten nach der Operation gebessert wahrend 29% unverandert blieben oder sich verschlechterten Wegen mangelnder Besserung wurden 42 Patienten nach verschiedenen Methoden nochmals operiert wobei bei der Halfte der Falle erneut Ultraschallbestrahlung verwendet wurde Das Horvermogen verschlechterte sich in 40% der Fälle was auch mit der langen Beobachtungszeit in Zusammenhang steht Die kalorische Erregbarkeit verminderte sich bei 70% der Patienten Bei den Patienten t deutlich verminderter kalorischer Erregbarkeit hatte h der Schwindel abgenommen Vorubergehende Fasparese bei vier Patienten war die einzige Komplika on

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THE NYSTAGMUS THRESHOLD IN TURNING TEST IN DIFFERENT AGE GROUPS AND IN PATIENTS SUFFERING FROM OTOSCLEROSIS

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Abstract The threshold of nystagmus during angular acceleration and deceleration was investigated in two series of healthy persons with an average age of 20 and 42 years and in series of otosclerotic patients with an average age of 42 years It was shown that age as such does not influence the thresholds but a lesion in the inner ear such as otosclerosis causes higher thresholds even in younger subjects

The use of the modern rotating chair provides us with the possibility of producing very small accelerations and decelerations, while simultaneous recording of the ENG makes it possible to detect small alterations in the nystagmus

Hilding (1953), de Vries & Schierbeek (1953) as well as Roggeveen & Nijhoff (1956) have shown that the threshold of the minimum perceptible acceleration and deceleration varies from 0 25°/sec² to 3 5°/sec² According to Montandon & Russbach (1956) the threshold of the nystagmus and sensation of turning in normal subjects approximates 0 8°/sec², a value which applies both to acceleration and deceleration A somewhat lower threshold 0 4°/sec² approximately has been reported by Decher (1965)

In the present investigation the threshold of acceleration and deceleration has been determined in two series of normal subjects and in otosclerotic patients before operation

MATERIAL

Three groups of subjects were investigated The first series consisted of 20 female students aged 19 to 22 from the local Nursing School The mean age of this group was 20 years The second series consisted of 20 female nurses and assistant nurses aged 36 to 50 from the Otolaryngological Clinic, the mean age being 42 years All members of these two groups had normal hearing Subjects with a history of vertigo or dizziness, as well as subjects with neurological disorders or diseases of the ears were excluded The third series consisted of 60 patients (31 female, 29 male) suffering from clinical otosclerosis, aged 18 to 64 the mean age being 42 years The airbone gap was approximately 27 dB in average and bone-conduction values were correspondingly 15 dB (in 250, 500, 1000, 2000 and 4000 cps) After examinations the patients in the series were all operated on (at the Otolaryngological Clinic, during 1970–71) and the final diagnosis was verified at the operation

METHODS

Rotatory tests were carried out by using the rotating chair of Polman (Mod II & III) Chlorided silver e trodes (7 mm in diameter)

Table I *The thresholds of angular acceleration and deceleration during rotatory stimulation (expressed in degrees/sec²)*

	Series I Healthy subjects Mean age 20 years (19–22 years)		*Series II* Healthy subjects Mean age 42 years (36–50 years)		*Series III* Patients suffering from otosclerosis Mean age 42 years (18–64 years)	
	deg/sec²	S D	deg/sec²	S D	deg/sec²	S D
Acceleration to the right	0 35	0 13	0 37	0 13	0 49	0 33
Acceleration to the left	0 32	0 11	0 32	0 10	0 60	0 28
Deceleration to the left	0 31	0 04	0 33	0 07	0 58	0 33
Deceleration to the left	0 30	0 00	0 37	0 12	0 50	0 28

were used Recording electrodes were located on the skin near the temporal canthi of the two eyes and an earthed electrode was placed on the skin of the forehead The electrodes were attached to the skin with adhesive tape and electrode jelly was used to improve the contact A condenser coupled amplifier, time constant 2 seconds, was used All recordings were performed in a dark room with the subjects eyes closed The subject to be examined was in sitting position The head of the subject was fixed to a forward declining position making an angle of 30° from the vertical position

The threshold of acceleration was measured by accelerating for 90 seconds at the rate of 0 2°/sec² to the right The subject was then rotated at a constant speed for 120 seconds and subsequently decelerated at the rate of 0 2°/sec² (acceleration to the left) Two minutes later the same procedure was repeated now in the reversed direction This procedure was continued with a stepwise increase at 0 2°/sec² of acceleration and deceleration until measurable nystagmus was observed

RESULTS

The results are given in Table I In series I consisting of young healthy subjects with an average age of 20 years (19–20 years) the threshold of acceleration to the right was 0 35°/sec² and to the left 0 32°/sec² The thresholds of deceleration were correspondingly 0 31°/sec² to the right and 0 30°/sec² to the left These thresholds are equal In series II, consisting of older, healthy subjects with an average age of 42 years (36–50 years) the corresponding values were 0 37°/sec² for acceleration to the right and 0 32°/sec² to the left and 0 33°/sec² for deceleration to the right and 0 37°/sec² to the left Also these thresholds are equal

There are no statistically significant differences in thresholds between sides In series III, consisting of patients suffering from otosclerosis with an average age of 42 years (18–64 years) the threshold of acceleration to the right was 0 49°/sec² and to the left 9 60°/sec² The corresponding values of deceleration were 0 58°/sec² and 0 50°/sec² Also these small differences in thresholds between right and left are statistically not significant This III series was devided in two subseries The first one of these consisting of 20 patients with a mean age of 29 years (18–35 years) and the second one consisting of 40 patients the mean age being 48 years (35–64 years) The thresholds of these both subseries were equal to those of the III series

COMMENTS

It is obvious (Table I) that there are no significant differences in thresholds of acceleration and deceleration to right and left be

tween the two age groups of healthy persons The statistically comparison of thresholds of acceleration and deceleration between series I and series III and between series II and series III The results show that the thresholds of acceleration and deceleration of patients suffering from otosclerosis are all statistically significantly higher than the thresholds of healthy subjects (P 0 01) There are some earlier investigations where they have shown that the senile vestibular apparatus has a diminished reactivity to rotational as well as caloric stimulus (Camarada & Lumia, 1959, Zelenka & Slaninova, 1964, Rossberg, 1964, Haas, 1964) On the contrary Forgacs (1957) reported ' normal ' vestibular reactions in all cases

The aim of this study was to compare the thresholds of younger and older people and the thresholds of patients suffering from otosclerosis

It was interesting to observe that there were no differences in thresholds between the two first series, but statistically very significant differences between both healthy and otosclerotic groups It can thus be concluded that age as such does not influence the thresholds On the other hand such a disease as otosclerosis, which affects primarily the labyrinthine capsule and the round transmitting system but also semicircular canals (Bretlau & Jorgensen 1968 Chevance et al 1969) causes higher thresholds of angular acceleration and deceleration (Virolainen 1972)

ZUSAMMENFASSUNG

Es wurde die Schwelle des Nystagmus bei zunehmender und abnehmender Winkelbeschleunigung untersucht und zwar anhand von zwei Gruppen gesunder Personen mit einem Durchschnittsalter von 20 und 4? Jahren und einer Gruppe von Patienten mit Otosklerose und mit einem Durchschnittsalter von 42 Jahren Es wurde gezeigt dass das Alter keinen Einfluss auf die Schwelle hat dass aber eine Storung im Innenohr in diesem Fall Otosklerose sogar bei jungeren Patienten hohere Schwellen verursacht

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RADIOSIALOMETRY

I A Method for Evaluation of Salivary Gland Disorders

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Abstract The temporal course of the total $^{99}Tc^mO_4$ accumulation in the parotid gland after fast intravenous injection of the radionuclide is studied by examination with quantitative radiosialometry The method adopted is based on the use of two collimated symmetrically located and opposite placed NaI-detectors The normal ranges of five evaluation parameters are obtained from a control material of 100 parotid glands in 50 individuals without disorders of the parotid glands The reproducibility of these five evaluation parameters is estimated and the sensitivity and the detectability of the parameters as well as different sources of variation are discussed Classification boundaries between normal and abnormal values are arbitrarily chosen from the normal ranges

Diagnostic methods employed in salivary gland disorders are mainly based on the examination either of the morphology or the function of the pathologic glands The morphology is examined by various methods such as careful palpation sialography fine needle aspiration biopsy or excision biopsy The function of the pathologic glands is commonly evaluated from the examination of the salivary flow by different sialometric methods or by the chemical analysis of the saliva composition Pathologic processes engaging the salivary glands may cause an abnormal salivary flow or abnormal concentrations of for instance, Na K Ca protein or amylase However, chemical analysis of the saliva is of little diagnostic value because of the wide ranges of normal values caused by many different factors, such as variations in blood concentrations and salivary secretion rate Evaluation of sialometric values is also of limited value in diagnostic work due to the wide normal variation in the salivary flow

Obviously, there is a need for several different methods in the diagnosis of disorders of the salivary glands Scintigraphy and radiosialometry are methods which offer further possibilities in evaluating pathologic changes in the salivary glands These methods differ in principle both from morphologic examination and examination of the salivary flow or saliva composition They are based on the fact that normal salivary gland tissue actively transports $^{99}Tc^mO_4$ from plasma to saliva concentrating the radionuclide 5–50 times by metabolic processes of the salivary gland cells (Harden et al 1968) It can be presumed that diseased glands with pathologic disturbances of the metabolism have a decreased capacity to transport $^{99}Tc^mO_4$ from plasma to saliva against the high concentration gradients In these pathologic salivary glands the accumulation of $^{99}Tc^mO_4$ in the salivary gland tissue and in the saliva can be expected to decrease Thus different disorders of the salivary glands can be diagnosed by the examination of the $^{99}Tc^mO_4$ accumulation process

Scintigraphic examination offers both morphologic and functional aspects of local or general pathologic processes within the parotid glands (Schall & Di Chiro 1972) There are however, considerable normal var-

Fig 1 Age and sex distributions in the control group A

iations in scintigraphic results, partly because of methodological errors and the subjective evaluation of the salivary gland scintigrams obtained (Enfors et al , 1969)

The aim of this study was to develop radiosialometry (Lind & Söderborg, 1971, Eneroth et al , 1972) into an uncomplicated and reliable method for the diagnosis of parotid gland disorders in clinical praxis by analysing different evaluation parameters, their normal ranges and different sources of variation The method is based on the quantitative examination of the temporal course of the total $^{99}Tc^{m}O_4$ accumulation in the parotid glands after fast intravenous injection of the radionuclide

CASE MATERIAL

The subjects studied consisted of 24 medical students, nurses and ward maids and 32 patients with diseases considered not to affect the salivary glands (for instance cancer of the uterus, deviation of the nasal septum etc) Six of the total 56 cases were excluded by mishaps in the measurements Thus 2 cases were excluded because the radionuclide injection was not completely intravenous and 4 cases because the salivary flow was not sufficiently inhibited The remaining 50 individuals were divided into five groups (A–E)

Group A All 50 individuals Age and sex distributions are given in Fig 1

Group B All 15 male individuals in group A (15)

Group C 15 female individuals in group A, closest in age to the males in group B

Group D All individuals in group A, aged between 18 and 30 (27)

Group E All individuals in group A aged between 60 and 80 (10)

Furthermore, 15 individuals with neoplastic or inflammatory diseases of the parotid glands were examined (*group F*) and another 11 individuals without diseases expected to influence the $^{99}Tc^{m}O_4$ distribution in the body (*group G*)

METHOD

The method was based on the principles earlier described by Lind & Soderborg in 1971 and Eneroth et al in 1972 In the present study, the measuring performance was simplified

1 0 mg atropinesulphate was administered by intramuscular injection about 15–30 min before the injection of $^{99}Tc^{m}O_4$ The patient was placed supine and told not to move his tongue or mouth Both buccal cavities as well as the floor of the mouth were filled by three compresses 1 0–2 0 mCi $^{99}Tc^{m}O_4$ was administered by fast intravenous injection and the registrations were started immediately Two collimated detectors (NaI 2"×2", collimator length 35 cm, aperture circular $\varnothing$=5 cm) were used (Fig 2) The detectors were

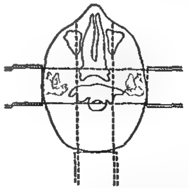

Fig 2 Arrangement of the detectors The measuring region of the right and left detectors includes both parotid glands The measuring region located between the parotid glands is measured from the neck

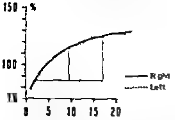

Fig 3 Measuring values obtained from the right and left detectors between 0–24 minutes after the injection of $^{99}Tc^{m}O_4$ One individual of the control group A

symmetrically placed opposite each other with the common central axes through the parotid glands They registered simultaneously the same region of the skull from the right and left sides, respectively The circular apertures, close to the skull, touched the mandibular angles from above and covered the lateral projections of the parotid glands (Fig 2)

14–20 registrations (measuring time 60 sec) were made automatically with 15 sec intervals and the obtained values were punched on paper tape The two compresses placed against the parotid ducts were removed immediately after the final registration and placed in thin plastic cups within the apertures f the collimators Their radionuclide content s measured

The results obtained were computed by a PDP-8 computer and all values were corrected for imbalance between the detectors, and for radioactive decay The correction factors for imbalance were obtained from measurements of a plastic vessel containing a homogeneous solution of $^{99}Tc^{m}O_4$ in water These measurements were repeated immediately before each patient examination (Larsson et al , 1975) A mean value of the 3rd 4th and 5th values for both sides (six values) was calculated and used as a reference All values were then calculated in per cent of this reference value, and the change with time was illustrated by graphically smoothed curves (Fig 3) The increases between the 2nd to 8th, 8th to 14th and 2nd to 14th values, calculated from these curves, were obtained in per cent of the reference value Because of the 15 second intervals between each 60 sec measuring period, the corresponding mean times were 1¾–9¼ min, 9¼–16¾ min and 1¾–16¾ min, respectively The differences between the right and left side increases (1¾–9¼ min and 1¾–16¾ min, respectively) were also calculated and obtained in per cent of the reference value (Fig 3)

The $^{99}Tc^{m}O_4$ content in a region between the parotid glands was measured with one of the detectors equipped with a narrower collimator (length 30 cm, aperture square, 3×3 cm) The aperture was located close to the neck and the detector's central axis was located in the median plane between the parotid glands (Fig 2) The measuring values obtained were calculated in the same way in per cent of the mean value of the obtained 3rd, 4th and 5th values and the changes between the 2nd to the 8th, 8th to 14th and 2nd to 14th values were calculated from graphically smoothed curves

RESULTS

After fast intravenous injection of $^{99}Tc^{m}O_4$ there was an accumulation of the radionuclide in the measuring region containing both parotid glands The accumulation process during the first minute was not registered in detail The accumulation between 1¾ and 9¼ min was

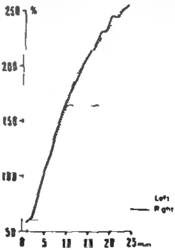

Fig 4 Measuring values in the right and left detectors obtained in one individual after a partially subcutaneous injection of $^{99}Tc^{m}O_4$

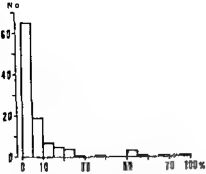

Fig 5 Amounts of $^{99}Tc^mO_4$ in compresses covering the parotid gland ducts given in per cent of the reference values used Values of 108 parotid glands from 54 individuals

generally larger than that between 9¾ and 16¾ min after the injection of $^{99}Tc^mO_4$ Generally, both sides increased their $^{99}Tc^mO_4$ content symmetrically (Fig 3)

If the $^{99}Tc^mO_4$ injection in error was given subcutaneously, the accumulation within the measuring region was symmetric, but exceeded by far the normally obtained values between 2 and 20 min (Fig 4) Accidental subcutaneous injection was thus possible to recognize from the shape of the curves obtained

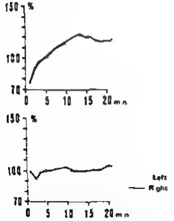

Fig 6 Two cases with losses of $^{99}Tc^mO_4$ from the parotid glands to the compresses covering the parotid ducts The accumulation of $^{99}Tc^mO_4$ in the parotid glands might cease after some time (upper curve) or not occur at all (lower curve) The amounts of $^{99}Tc^mO_4$ in the compresses covering the parotid ducts were in these cases large i e between 50–70 per cent of the reference value used

Table I *Mean values and standard errors (S E) inter-individual and intra individual standard deviations (S D) of the evaluation parameters used*

	Mean	Inter individual S D	S E	Intra individual S D
Increase right side				
1¾–9¾ min	27.9	9.3	1.3	2.3
9¾–16¾ min	11.0	7.2	1.0	2.1
1¾–16¾ min	39.0	15.5	2.2	4.1
Increase left side				
1¾–9¾ min	28.6	10.0	1.4	2.6
9¾–16¾ min	12.7	7.3	1.0	2.3
1¾–16¾ min	41.3	16.4	2.3	4.5
Side difference				
1¾–9¾ min	−0.6	2.3	0.3	3.2
1¾–16¾ min	−2.2	4.7	0.7	3.9

The values of the $^{99}Tc^mO_4$ content in the compresses covering the orifices of the parotid ducts were calculated in per cent of the reference value defined above, and were in general less than 25% (Fig 5) High values indicated a loss of $^{99}Tc^mO_4$ from the measuring region owing to saliva leakage The accumulation was then decreased and the curves obtained appeared abnormal in some cases (Fig 6)

The amount of radionuclide in the measuring region located between the parotid glands was measured in individuals in group G and found to be almost constant between 1¾ and 16¾ min of the injection There was on an average a small increase between 1¾ and 9¾ min after injection (+0.8±4.7%) and a small decrease between 9¾ and 16¾ min (−3.4±3.3%) and thus a small decrease between 1¾ and 16¾ min (−2.6±7.1%)

The measuring values from the measuring region containing both parotid glands increased and these increases were calculated for all individuals The results obtained are presented in Table I and Figs 7–9

The differences between the right and left sides were also calculated for all individuals in group A The side difference was arbitrarily given a positive sign if the increase of the right

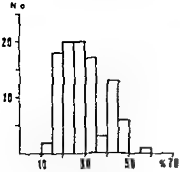

Fig 7 Distribution of values of the evaluation parameter Increase 1¾–9¼ minutes Control group A

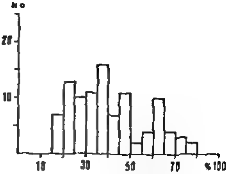

Fig 8 Distribution of values of the evaluation parameter Increase 1¾–16¾ minutes Control group A

side was largest, and a negative sign if the increase of the left side was largest Obtained values are presented in Table I and Figs 10–11 The mean value of side differences (−0 6%) obtained during the measuring time, 1¾–9¼ min did not deviate significantly from 0 *The values were almost symmetrically distri-*buted around their mean value and the variation was small (S D =2 3%) compared with the variation of corresponding increases (S D =9 3% and 10 0%, respectively) (Fig 10, Table I)

The mean value of side differences (−2 2%) tained during the measuring time 1¾–16¾ min deviated significantly ($p<0$ 05) from 0 (Fig 11 Table I) Hence, there was in this evaluation parameter a significant difference between the right and left sides with a higher accumulation of $^{99m}TcO_4$ at the left side

The distribution of increases in group B (males) and group C (females) corresponded well with each other as exemplified in Fig 12, and there was no significant difference between group B and C as tested by Wilcoxon's ranksum test

The distribution of increases in group D (age 18–30) and group E (age 60–80) corresponded well with each other, as shown in Fig 13 and there was no significant difference between group D and E in Wilcoxon's ranksum test

All the individuals in group F were examined twice within a few days and the intra-individual variations (within individuals) were calculated from the paired observations The intra-individual S D of the increase parameters was much smaller than the corresponding inter-individual (between individuals) S D , but the intra-individual S D of side

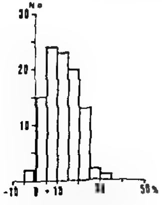

Fig 9 Distribution of values of the evaluation parameter Increase 9¼–16¾ minutes Control group A

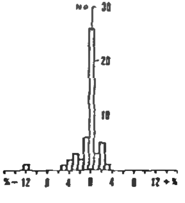

Fig 10 Distribution of values of the evaluation parameter "Difference 1¾–9¼ minutes Control group A

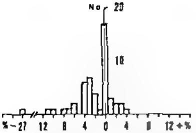

Fig 11 Distribution of values of the evaluation parameter Difference 1½–16½ minutes Control group A

difference parameters was of approximately the same magnitude as the corresponding inter individual S D (Table I)

DISCUSSION

General principles

The purpose was to design a simple method of high accuracy in the diagnose of salivary gland disorders for practical clinical use, i e the pathologic cases of interest to be separated from the normal cases with the highest possible reliability

Many pathologic processes of the salivary glands cause morphologic changes, which can be examined in order to obtain a diagnosis Several pathologic processes cause functional disturbances which also can be examined in order to separate the pathologic cases from the normal

The function of the parotid glands can be evaluated by principally different methods The salivary flow is measured by sialometric methods but large normal variations imply that only very small values can be assessed as abnormal (Enfors, 1962) The chemical composition of the saliva is changed by some diseases, but large normal variation makes the evaluation of results difficult (Rauch, 1959, Benedek Spät, 1973) The capacity of the parotid glands to accumulate $^{99}Tc^{m}O_4$ from plasma can be demonstrated by scintigraphy and measured by the radiosialometric method (*Lind & Soderborg, 1971, Eneroth et al*, 1972) The given amount of $^{99}Tc^{m}O_4$ (1–2 mCi) caused a whole body dose of about 0 01–0 04 rad, and a critical organ dose of about 0 5–1 rad (thyroid gland, salivary glands) (Smith, 1965, National Institute of Radiation Protection, Sweden, 1969)

The amount of $^{99}Tc^{m}O_4$ in tissues within the measuring region, but located between the parotid glands was found to be almost constant between 2 and 20 min of fast intravenous injection of the radionuclide The increase of $^{99}Tc^{m}O_4$ in the measuring region, which included both parotid glands (Fig 2) was thus caused by an accumulation of the radionuclide in the glands

The change with time of the total $^{99}Tc^{m}O_4$ content within the region was studied, and hence the temporal distribution of the radio-

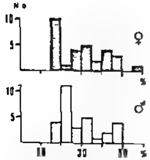

Fig 12 Distribution of values of the evaluation parameter Increase 1½–9½ minutes Group C (females) and group B (males)

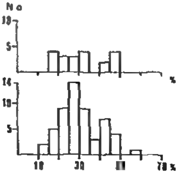

Fig 13 Distribution of values of the evaluation parameter Increase 1½–9½ minutes Group E age 60–80 (upper diagram) and group D age 18–30 (lower diagram)

nuclide instead of its spatial distribution was used as the basis for evaluation contrary to scintigraphy or the use of gamma cameras without computers

Sensitivity and detectability

The concepts of sensitivity and detectability have been defined elsewhere and found valuable in trials to optimize the symmetry detector method for gammaencephalography (Lind & Larsson 1975)

The sensitivity (S) of a parameter was defined as the true mean value of parameter units obtained per unit of the defined effect of interest (for instance counts/min per mCi $^{99}Tc^mO_4$ in the measuring region) The detectability (δ) of a parameter was defined as the smallest effect of interest which can be detected with a significance that corresponds to q standard deviations

$$\delta=\frac{q \cdot \sigma}{S}$$

where σ is the standard deviation of the parameter in a normal group

In order to increase the detectability (small values of δ) the standard deviation of u's σ of the parameter used should be as small as possible and the sensitivity S of the parameter as high as possible

An evaluation parameter can be constructed by combining several different measuring parameters Example side difference in this method was an evaluation parameter which was constructed by a subtraction of the increase of measuring values in the left detector from the corresponding increase of measuring values in the right detector The evaluation parameter was made relative by division with a reference value (=mean value of the 3rd to 5th measuring values of both detectors) Correspondingly large numbers of evaluation parameters can be constructed with different sensitivity different normal standard deviation and thus different detectability

The sensitivity of different evaluation parameters in different measuring situations and the physical characteristics of a symmetry detector method for gammaencephalography (stability of counting efficiency choice of pulse height discrimination detector response) have been described earlier and the obtained results were possible to apply on the two detectors used for radiosialometry (Larsson et al 1975) It was demonstrated that the sensitivity of evaluation parameters based on side difference was smaller than the sensitivity of evaluation parameters based on the increase of measuring values in one detector Despite this side difference parameters offered better detectability because of smaller standard deviation in a normal group (Lind & Larsson 1975)

In order to increase the detectability of the chosen evaluation parameters its standard deviation in a normal group should be decreased without decreasing the sensitivity Therefore different sources of variation were analysed and reduced within practical limits

Sources of variation

Variation caused by counting statistics (15000–60000 cpm) general background (100–200 cpm) or by the electronic device have elsewhere been demonstrated to be negligible (Larsson et al 1975)

The position of detectors spatial distribution of the radionuclide change of radionuclide distribution with time salivary flow age sex and individual biologic variation of the parotid glands are the more important sources of variation

The position of the detectors constituted one source of variation which may prove considerable A small parotid gland will not extend outside the measuring region even if there are small changes in the detectors positions (head movements wrong localization of the detectors) but large glands might extend outside the measuring region and the parotid accumulation of $^{99}Tc^mO_4$ is then not registered completely Probably the main clinical interest will be directed to small or very small accumulations and hence this source of variation is

less important in practical clinical work. The positions of the detectors were clearly defined: the collimators touched both zygomatic arches and inhibited head movements. The intra-individual variations of increase parameters were found small, which indicated that the change of the detectors' positions between two examinations was only a small source of variation. If the measuring region was extended, the relative size of parotid gland accumulation of $^{99}Tc^{m}O_4^-$ should decrease compared to the larger amount and enlarged variation of $^{99}Tc^{m}O_4^-$ in the measuring region. In addition parts of the submandibular glands or brain might be more or less included in the measuring region and thus cause further variation. Nevertheless, it may be desirable to increase the diameter of the collimator tubes from 5.0 to 6.0 cm in order to assure the inclusion of the parotid glands within the measuring region.

The spatial distribution of $^{99}Tc^{m}O_4^-$ varies because of different blood volume, hemoglobin content, $^{99}Tc^{m}O_4^-$ distribution between blood, thyroid gland, ventricular mucosa, kidneys, etc. Most of this variation can be avoided by the use of relative evaluation parameters, i.e. each measuring value is related to a reference value which compensates for this variation, for instance the plasma concentration at a certain time after the injection, as described earlier (Lind & Söderborg 1971). The less complicated use of the mean value of the 3rd, 4th and 5th measuring values is preferable in a method intended for clinical work.

The change of $^{99}Tc^{m}O_4^-$ concentration with time was dependent on many factors. The relative change of the plasma concentration of $^{99}Tc^{m}O_4^-$ between 2 and 70 min of fast intravenous injection was earlier found to be reproducable between individuals within narrow limits (Lind & Söderborg 1971). However, the injection technique may fail and if a sufficiently large amount of radionuclide was injected subcutaneously, the registered amount of radionuclide within the measuring region gradually increased; not because of accumulation in the parotid glands, but because of the changed course of plasma concentration. Two of 56 individuals initially examined were excluded because of subcutaneous injection, recognized from the abnormally large increase of the registered amount of radionuclide (Fig. 4). It was found advisable to control the position of the injection needle regularly by immediate aspiration of some blood both before and after the injection further to assure a correct intravenous administration of $^{99}Tc^{m}O_4^-$.

In order to estimate the variation caused by the change with time of the $^{99}Tc^{m}O_4^-$ content in the tissues of the measuring region outside the parotid glands, measurements were made with the central axis of one detector located in the median plane between the parotid glands (Fig. 2). The obtained results from group G demonstrated that the amount of $^{99}Tc^{m}O_4^-$ in tissues between the parotid glands was in mean almost constant between $1\frac{3}{4}$ and $16\frac{3}{4}$ min after injection. There was a not negligible variation between the examined individuals, but it was regarded as too small to motivate the economic and practical disadvantages in clinical praxis of using three detectors simultaneously. However, for scientific purposes it should be of great value to achieve control of the temporal changes of the $^{99}Tc^{m}O_4^-$ concentration in the measuring region outside the parotid glands.

The salivary flow has elsewhere been demonstrated to constitute one important source of variation in salivary gland scintigraphy (Enfors et al. 1969). The accumulation of $^{99}Tc^{m}O_4^-$ in the parotid glands is dependent on the total transport of $^{99}Tc^{m}O_4^-$ from plasma into the gland and saliva and on the salivary transport of $^{99}Tc^{m}O_4^-$ out from the glands (Lind & Söderborg 1971, Ancri et al. 1973). There is a large variation between individuals of both the resting and the stimulated salivary flow (Enfors 1962) and therefore any salivary transport of $^{99}Tc^{m}O_4^-$ out from the glands constitutes an important source of variation as

demonstrated by Fig 6 These losses of $^{99}Tc^mO_4$ were however easily controlled by the inhibition of the salivation and measurements of the actual losses 1 0 mg atropinesulphate, administered by intramuscular injection, caused a pharmacological inhibition of saliva tion, and compresses in the oral cavity inhibited involuntary movements of the tongue and the floor of the mouth, which might press saliva out from the glands Accidental transport of $^{99}Tc^mO_4$ from the glands to the oral cavity, during the examination was easily discovered by measuring the radionuclide content of the compresses, placed in the buccal cavities, against the parotid ducts

In general there was only a small amount of $^{99}Tc^mO_4$ in the compresses, but in a small number of cases their contents of $^{99}Tc^mO_4$ were considerable (Fig 5) In order to reduce this source of variation, it was decided to exclude all examinations with a $^{99}Tc^mO_4$ con tent in one or two buccal compresses, higher than 25 per cent of the reference value Four of the initially examined 56 individuals were excluded for this reason It is evident that ontrolled salivation constitutes a source of riation, which also should make the in terpretation of salivary gland scintigraphy un certain (Enfors et al 1969)

Age and sex were no important sources of variation Females and individuals aged 18–30 years were over represented in the material examined However there was no difference in $^{99}Tc^mO_4$ accumulation between the group of oldest and youngest individuals (Fig 13) nor between males and females (Fig 12) The number of examined individuals was too small to permit definite conclusions concerning the influence of age and sex on the parotid gland capacity to accumulate $^{99}Tc^mO_4$ but large influences can be excluded However, very old or senile individuals and children were not examined

Individual biologic variation of the parotid glands is probably large as despite reductions of different sources of variation the values of increases varied considerably between individuals (Table I, Figs 7–9) It is to be supposed that this variation depends on the varying size as well as the varying functional capacity of the parotid glands, in accordance with the large variation of sialometry values obtained between normal individuals (Enfors, 1962, Ericson, 1974) The intra individual variation is much smaller (Table I), indicating that most methodological errors were sufficiently reduced It is possible to estimate the sizes of the parotid glands by sialography, and this part of normal biological variation can be reduced by dividing obtained results by the estimated size of the examined gland (Ericson, 1974) For ethical and practical reasons it was not possible to examine the volunteers of group *A* both by radiosialometry and sialography, and the relative importance of glandular size as a source of variation was thus not possible to estimate from this material

The variations of side difference parameters were much smaller than corresponding values of increase parameters (Table I, Figs 7–11) which indicated that the biological variation of glandular size and functional capacity are symmetric as well as the normal variation of $^{99}Tc^mO_4$ concentration with time in tissues within the measuring region but outside the parotid glands Besides, variation caused by the accidental occurrence of parotid gland tissue outside the measuring region was also assumed to be mainly symmetrical

The symmetry of the glands was great and the intra-individual variation (within individu als) of values of difference was of the same magnitude as corresponding inter individual variation (Table I) This indicated that methodological sources of variation (for in stance stabilization of salivary flow) become important in relation to the small biologic variation between the right and left parotid glands

The sensitivity of side difference parameters is smaller than the sensitivity of increase parameters (Larsson et al , 1975) However, the standard deviations of the side difference parameters obtained from a control group

Table II *Classification boundaries between normal and abnormal The values were arbitrarily chosen from the normal ranges*

	I Definitely normal (%)	II Probably normal (%)	III Probably abnormal (%)	IV Definitely abnormal (%)
Increase values				
1¾–9¾ min	>+18	+12 – +18	+8 – +12	<+8
1¾–16¾ min	>+20	+15 – +70	+10 – +15	<+10
Side difference values				
1¾ 9¾ min	3 – +2	6 – −3 +2 – +4	9 – −6 +4 – +7	<−9 >+7
1¾–16¾ min	−5 – +2	10 – −5 +2 – +4	−15 – 10 +4 – +8	<−15 >+8

where much smaller than corresponding standard deviations of the increase parameters (Table I) Therefore, the detectability concerning unilateral changes of the $^{99}Tc^mO_4$ accumulation in the glands was supposed to be better in side difference parameters than in corresponding increase parameters

Classification of results

In diagnostic work the obtained measuring results must be classified as normal or abnormal and it is often practical to use the following classification of results

I Definitely normal
II Probably normal
III Probably abnormal
IV Definitely abnormal

If normal values have a gaussian distribution confidence intervals with different levels of probability are easily defined However the values of increase were not symmetrically distributed around their mean values and the number of observations was too small to permit definition of confidence intervals For the purpose of diagnosing pathologic lesions of the glands with few exceptions only the decreased capacity to accumulate $^{99}Tc^mO_4$ is of interest Therefore borders between normal and abnormally low values were chosen arbitrarily, from the distributions in Figs 7–9 The chosen borders are presented in Table II The borders of classification of side difference values were arbitrarily chosen from Figs 10 and 11 (Table II)

Diagnostic accuracy

The concepts of detectability and sensitivity defined above were of great value in the optimal choice and design of evaluation parameters intended for the detection of an abnormal radionuclide distribution in certain individuals The knowledge obtained concerning the normal ranges of the evaluation parameters investigated is necessary in assessing examination results of unknown individuals as normal or abnormal

However the purpose of a diagnostic method is not to separate individuals with abnormal radionuclide distributions from the normal but to offer high diagnostic accuracy in certain diseases i e high capacity to separate the pathologic cases of interest from normal cases The diagnostic accuracy of the method is not only dependent on the detectability of the evaluation parameters used but also on the correlation between the disease of interest and the evaluated radionuclide distribution Therefore, the diagnostic accuracy of the described evaluation parameters in radiosialometry must be studied by compari-

son between examination results obtained from normal individuals and results obtained from individuals with different verified parotid gland diseases (Eneroth & Lind, 1975)

ZUSAMMENFASSUNG

Der zeitliche Verlauf der totalen ^{99}Tc^{m}O$_4$ Anreicherung in der Gl parotis nach schneller intravenoser Injektion des Radionuklids wurde mit quantitativer Radiosialometrie untersucht Bei dieser Untersuchungsmethode bedient man sich zweier mit entgegengesetzt aus gerichteten Kollimatoren versehener symmetrisch an gebrachter und gegenuberliegend plazierter NaI Detektoren Die normalen Bereiche der funf benutzten Auswertungsparameter wurden aus einem Kontroll material von 100 Parotisdrusen von 50 Personen ohne Erkrankungen der Parotisdrusen erhalten

Die Reproduzierbarkeit dieser funf Auswertungsparameter wird abgeschatzt und die Empfindlichkeit und Nachweisbarkeit der Parameter sowie verschiedene Ursachen der Streuungen werden diskutiert Die Klassifikationsgrenzen zwischen normalen und abnormen Werten werden ausgehend von den Werten der 50 Normalpersonen definiert

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RADIOSIALOMETRY

II. The Diagnostic Accuracy of Different Evaluation Parameters

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Abstract. The temporal course of $^{99}Tc^{m}O_4$ accumulation in the parotid gland after fast intravenous injection of the radionuclide was measured quantitatively by radiosialometry in 32 patients suffering from inflammatory or neoplastic diseases of the parotid glands. The diagnostic accuracy of five different evaluation parameters was compared. It was found that without reducing diagnostic accuracy the method used could be simplified by omitting three of the five evaluation parameters. This implies a shortening of the measuring time from 18 to 10 minutes. The diagnostic accuracy of radiosialometry in 14 cases with a general engagement of the parotid parenchyma (irradiation damage, collagen sialosis and chronic recurrent parotitis) was high. Thus all these 14 cases had abnormal radiosialometric values. Parotid tumours not causing extensive destruction of the parotid parenchyma were not detected by this method.

Normal parotid glands accumulate $^{99}Tc^{m}O_4$ from plasma. This accumulation can be examined by scintigraphic or radiosialometric methods (Borner et al., 1965; Lind & Soderborg, 1971; Eneroth et al., 1972 b; Eneroth & Lind, 1975). Radiosialometry examines the temporal course of $^{99}Tc^{m}O_4$ accumulation in the parotid glands. It is assumed that pathologic disturbances of the parotid metabolism causes a decrease of the accumulation of $^{99}Tc^{m}O_4$ in diseased glands.

The radiosialometric method was described by Lind & Soderborg (1971) and Eneroth et al. (1972 b) and has been simplified in order to increase its practicability in clinical use (Eneroth & Lind, 1975). Thus the accumulation of the radionuclide in the parotid glands was measured by two opposite symmetrically placed collimated detectors, and the temporal course of the accumulation process was evaluated from the percentual increases of registered activity in each detector as well as from the side differences between these percentual increases. Five different evaluation parameters as well as different sources of variation were analysed in order to gain high detectability concerning the $^{99}Tc^{m}O_4$ accumulation in the parotid glands (Eneroth & Lind, 1975). The normal ranges of these five different evaluation parameters were obtained by analysis of a control material consisting of 100 parotid glands (50 individuals).

The purpose of the present study is to estimate the diagnostic accuracy of the five evaluation parameters by a correlation study between a control group and a group of patients with parotid gland disorders. This was done in order to achieve a further simplification of the method by the exclusion of unnecessary evaluation parameters, though still without a reduction of the diagnostic accuracy of the radiosialometric method.

MATERIAL

The present study is based on the examination of 82 individuals. The case material is divided into six groups (A, B, C, D, E and F).

Group A includes 50 individuals without

diseases expected to affect the parotid glands and is therefore noted as the control group

Group B includes 5 patients irradiated for malignant tumours in the head and neck region Three patients were irradiated because of nasopharyngeal carcinomas In these cases, both parotid glands were included completely within the irradiated region and all the parenchyma of the 6 parotid glands received irradiation doses exceeding 6000 rads The remaining two patients suffered from carcinomas of the left bucca In these two cases, the left parotid glands were supposed to be within the radiation field, but small parts of the parotid parenchyma might extend beyond the irradiated region The right parotid glands received doses of less than 100 and 500 rads, respectively

Group C includes 4 patients with the following criteria of collagen sialosis subjective xerostomia and abnormally low salivary flow demonstrated by sialometry, bilateral sialoectasies demonstrated by sialography, keratoconjunctivitis sicca, and positive tests for rheumatoid factors (RF) and/or antinuclear factors (ANF) All these patients were women and at the examination their ages varied from 36–78 years

Group D includes 5 patients with chronic recurrent parotitis aged between 40 and 61 years (4 women and one man) All these patients had had relapses of parotid swelling over a long period These patients had bilateral symptoms, but two of the 5 patients had exclusively left sided symptoms In all 5 patients there were abnormal sialograms of the parotid glands with sialoectasies and strictures of the excretory ducts

Group E includes 14 patients with benign parotid tumours on either the left or right side sized 0 5×1 cm to 5×6 cm Twelve of the tumours were histologically classified as pleomorphic adenoma and two as papillary cystadenolymphoma (Warthin s tumour)

Group F includes 4 patients with one sided malignant parotid tumours In two cases the tumours were classified as highly differentiated mucoepidermoid carcinoma (2× cm and 3×3 cm, respectively) One tumo was a 2×2 cm large metastasis of squamo cell carcinoma and the remaining tumour w a very low differentiated mucoepidermoid ca cinoma growing invasively through the who left parotid gland

METHOD

The principles and the development of th radiosialometric method has previously bee described by Lind & Soderborg (1971), Eneroth et al (1972*b*) and Eneroth & Lind (1975 The patients salivary flow was inhibited b atropinesulphate and compresses in the or cavity Technetium pertecnetate ($^{99}Tc^{m}O$ was then administered by fast intravenous i

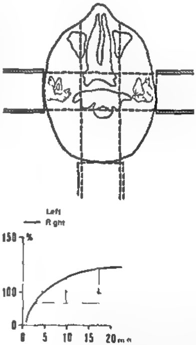


Fig 1 Arrangement of the detectors The measuring re gion of the right and left detectors includes both paroti glands The measuring region located between the paroti glands is not used in this study

The diagram demonstrates the measuring values of th right and left detectors from one patient suffering from low differentiated carcinoma invading the entire le parotid gland The accumulation of $^{99}Tc^{m}O_4$ in the righ unaffected gland is within normal limits but there is n accumulation of $^{99}Tc^{m}O_4$ between 2 and 20 minutes in th diseased gland

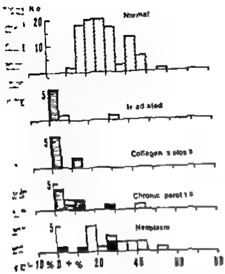


Fig 2 Distributions of values of the evaluation parameter Increase 1¾–9¼ min obtained from 164 parotid glands ... There were 50 control and ... (Group

neoplasms (Groups E and F) The light dotted piles indicate glands which could not be established to be completely located within the radiation fields glands without symptoms of chronic recurrent parotitis and glands with benign neoplasm (Group E) respectively Thus the dark piles indicate glands completely located within the radiation field glands with symptoms of chronic recurrent parotitis and malignant neoplasms respectively

jection and the accumulation of $^{99}Tc^{m}O_4$ in the parotid glands was measured by two collimated detectors placed opposite each other on a common axis (Fig 1) The temporal course of the accumulation process was evaluated by five evaluation parameters These were the three increase parameters, the percentual increases of registered activity in each detector between 1¾–9¼ min, 9¼–16¾ min and 1¾–16¾ min, respectively, as well as the two side-difference parameters, the differences between the percentual increases of the right and left detectors (1¾–9¼ min and 1¾–16¾ min respectively) (Eneroth & Lind, 1975) The obtained results were classified as normal or abnormal in accordance with the classification boundaries (Table I) arbitrarily chosen in considering the normal distribution (Eneroth & Lind 1975)

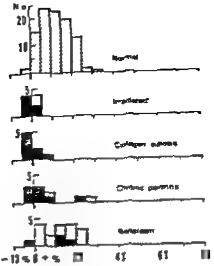


Fig 3 Distribution of values of the evaluation parameter "Increase 9¼–16¾ min" obtained from the same material and presented in the same way as in Fig 2.

RESULTS

Choice of evaluation parameters

The obtained values of each evaluation parameter are presented in Figs 2–6 Group B, C and D (patients with irradiated glands, collagen sialosis and chronic parotitis) deviated distinctly from the control group (A) in the evaluation parameters "Increase 1¾–9¼ min (Fig 2) and "Increase 1¾–16¾ min" (Fig 4)

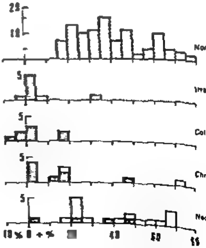


Fig 4 Distribution of values of the evaluation parameter Increase 1¾–16¾ min obtained from the same material and presented in the same way as

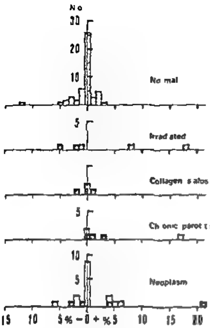


Fig 5 Distributions of values of the evaluation parameter 'Difference 1¾–9¼ min obtained from 164 parotid glands (82 individuals) There were 50 control individuals (Group A) 5 patients with heavily irradiated glands (Group B) 4 patients with collagen sialosis (Group C) 5 patients with chronic recurrent parotitis (Group D) and 18 patients with neoplasms (Groups E and F) The light dotted piles in dicate patients of which the irradiated gland could not be established as being completely located within the radia tion field or patients with unilateral symptoms of chronic recurrent parotitis or patients harbouring a benign neo lasm in one parotid gland (Group E) respectively The piles indicate patients with both glands completely ted within the radiation fields or patients with col lagen sialosis bilateral symptoms of chronic recurrent parotitis or malignant neoplasms respectively

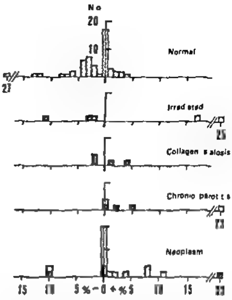


Fig 6 Distributions of values of the evaluation parameter Difference 1¾–16¾ min obtained from the same material as presented in the same way as in Fig 5

This separation was not distinct in the evalua tion parameters Increase 9¾–16¾ min", be- cause values for the control group were distri- buted from about −5 % to +35 % (Fig 3) The pathologic groups, B C and D were not sepa rated from the control group (A) by side differ- ence parameters ("Difference 1¾–9¼ min" and "Difference 1¾–16¾ min" in Figs 5 and 6), but two patients with unilateral irradiation damage of the parotid glands and one of two patients with anamnestic grounds for unilateral chronic parotitis had side difference values far beyond the normal range (Figs 5 and 6)

The results of group A (control group 50 cases) and group B, C and D gathered into one group (pathologic group, 14 cases) were clas- sified as normal or abnormal according to the classification scheme (Table I) Three differ ent classifications, based on three different combinations of evaluation parameters were made The first classification presumed that the examination of the $^{99}Tc^mO_4$ accumulation process was restricted to 10 min thus offering only the evaluation Parameters ' Increase 1¾–9¼ min ' and 'Difference 1¾–9¾ min The re- sults obtained in each of these two evaluation parameters were classified according to Table I and the patients examined were given the highest classification number (most abnormal) of either of these two evaluation parameters (Fig 7) The second classification was made presuming that the $^{99}Tc^mO_4$ accumulation pro cess examined was longer than 18 min and thus all evaluation parameters were obtain- able The results of each of the evaluation parameters "Increase 1¾–16¾ min" and "Dif ference 1¾–16¾ min" were classified according to Table I, and the patients examined, were given the highest classification number (most abnormal) of either of these two evaluation parameters (Fig 8) Finally, the third classifi-

Table I *Classification boundaries between normal and abnormal The values were arbitrarily chosen from the normal ranges (Eneroth & Lind, 1975)*

	I Definitely normal (%)	II Probably normal (%)	III Probably abnormal (%)	IV Definitely abnormal (%)
Increase values				
1¾–9¼ min	>+18	+12 – +18	+8 – +12	<+8
1¾–16¾ min	>+20	+15 – +20	+10 – +15	<+10
Side difference values				
1¾–9¼ min	−3 – +2	−6 – −3 +2 – +4	9 – −6 +4 – +7	<−9 >+7
1¾–16¾	−5 – +2	−10 – −5 +2 – +4	−15 – −10 +4 – +8	<−15 >+8

cation was based on the use of all five evaluation parameters except for "Increase 9¼–16¾ min", and the patients examined were given the highest number of classification (most abnormal) of either of these four evaluation parameters (Fig 9)

It is seen from Figs 7 and 8 that the combination of the two parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" (Fig 7) offered a better separation of the pathologic groups (B, C and D) from the control group (A), than the combination of the two parameters "Increase 1¾–16¾ min" and "Difference 1¾–16¾ min" (Fig 8) Further, it is seen from Figs 7 and 9 that the use of only the two evaluation parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" (Fig 7) offered a better separation of the pathologic groups from the control group than the use of all four evaluation parameters (Fig 9) It was concluded that the examination time could be restricted to 10 min without relevant reduction in diagnostic accuracy (i e capacity to separate pathologic cases from normal)

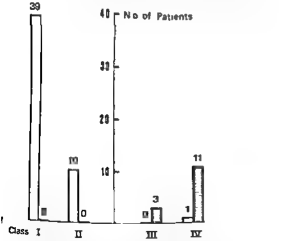
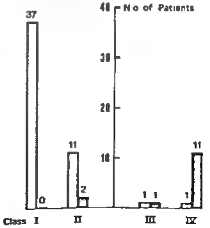


Fig 7 Classification of results as normal or abnormal according to Table I 40 control individuals (Group A light dotted piles) and 14 patients with chronic inflammatory reactions of the parotid glands (Group B C and D dark piles) Two evaluation parameters obtained from 10 minutes measurements were used as base for classification Increase 1¾–9¼ min" and Difference 1¾ 9¼ min



dark piles) Two evaluation parameters obtained from 18 minutes measurement were used as base for classification Increase 1¾–16¾ min and "Difference 1¾–16¾ min

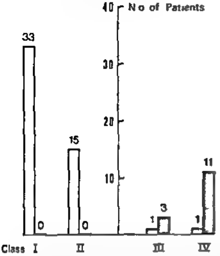


Fig 9 Classification of results as normal or abnormal according to Table 1 50 control individuals (Group A light dotted piles) and 14 patients with chronic inflammatory reactions of the parotid glands (Group B C and D dark piles) Four evaluation parameters obtained from 18 minutes measurement were used as base for classification Increase 1¾–9¼ min and Difference 1¾–9¼ min as well as Increase 1¾–16¾ min and Difference 1¾–16¾ *min*

Diagnostic accuracy

The clinical value of radiosialometry was therefore assessed from the diagnostic accuracy offered by merely the parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" It was then found that all patients irradiated because of nasopharyngeal carcinoma had increase values below 5% and side differences within the normal range Two patients irradiated because of a left-sided buccal cancer had increase values of the left, heavily irradiated side of 6% and 31% respectively (Fig 2) Both these patients had increase values of the right less irradiated side, well within the normal range and the side differences were clearly abnormal in both cases (Figs 2 and 5)

Three of four cases with collagen sialosis (Group C) had increase values between 0 and 5% and one case had values between 10 and 15% There were no side differences outside the normal range (Figs 2 and 5)

Four of five cases with chronic parotitis (Group D) had increase values of both parotid glands between 0 and 15% and one of five wit symptoms only of the left side had increas values within the normal range but the sid difference in this case was clearly abnorm with lower values of the affected left sid Another of these five cases had unilater symptoms, but her side difference was 0 (Fig 2 and 5)

Eight of 14 cases with benign tumour (Group E) were classified as definitely norm (Class I) No case was classified as definitel abnormal (Class IV), but three cases wer classified as probably abnormal (Class III) The increase values of both sides of one cas with a large pleomorphic adenoma (5×6 cm were normal (34 and 30%) and the differenc was only 4%, indicating that the total accumulation in the affected parotid gland was not o only slightly decreased

Two of four cases with malignant tumour (Group F) were classified as definitely norma or probably normal and two cases as probabl or definitely abnormal The definitely abnormal case (Increase right 22%, left 0%) had a low differentiated cancer invanding the lef gland (Fig 1)

DISCUSSION

Radiosialometry was developed in order to obtain an uncomplicated and reliable quantitative method for diagnosing pathologic lesions of the parotid glands (Lind & Soderborg, 1971, Eneroth et al , 1972, b) The method is based on the study of the temporal course of the $^{99}Tc^{m}O_4$ accumulation process in the parotid glands $^{99}Tc^{m}O_4$ is transported from plasma to the saliva by metabolic processes of the parotid gland cells and hereby concentrated 5–50 times (Harden et al 1968) It can be presumed that pathologic lesions of the parotid glands disturb these metabolic processes and hence the concentration capacity A study of the accumulation of $^{99}Tc^{m}O_4$ in the parotid glands after fast intravenous injection thus can be expected to offer possibilities to separate

glands with pathologic lesions from normal glands (Schall & DiChiro, 1972)

The original radiosialometric method Lind & Soderborg, 1971) has been developed and simplified (Eneroth & Lind, 1975) Five evaluation parameters and their normal ranges have been described as well as different sources of variation (Eneroth & Lind, 1975) As far as unilaterally decreased $^{99}Tc^{m}O_4$ accumulation is concerned evaluation parameters based on side difference were shown to have higher detectability than parameters based on the increase of registered activity in one detector (Eneroth & Lind, 1975) However, the diagnostic accuracy (i e capacity to separate pathologic cases of interest from normal) of the different evaluation parameters concerning parotid gland lesions depends on the correlation between the evaluated effect of interest (temporal course of the $^{99}Tc^{m}O_4$ accumulation) and the pathologic lesion of interest (for instance chronic inflammation)

Choice of evaluation parameters

The aim of the present study was to judge and compare the diagnostic accuracy of the five above mentioned quantitative evaluation parameters Therefore, patients with definite lesions of the parotid glands were examined

As neoplasms usually leave most of the parotid parenchyma unaffected, tumours were excluded from the patient material used to test and compare the diagnostic accuracy of the five evaluation parameters Instead, patients were chosen with parotid gland diseases expected to cause a generic engagement of the parotid parenchyma

As Eneroth et al (1972, a) pointed out, the parotid glands were usually extinguished after heavy irradiation In the present study, a total dose of 5 100–7 200 rads was given against eight parotid glands (five patients) located within the radiation beam The results obtained from these patients should be representative for glands with a generalised disorder of the parotid gland pa

The entire parenchyma of the eight parotid glands of the four patients with collagen sialosis was assumed to be seriously affected because the demonstrated pathologic influence on their morphology and function was definite (sialoectasies and reduced salivary flow) The five patients with chronic recurrent parotitis were also assumed to have seriously affected parotid parenchyma because of long periods of relapsing parotid swellings and definite pathologic sialographic findings However, relapsing infections might leave some parts of the parenchyma unaffected and two cases had only unilateral symptoms

The five different evaluation parameters, above described, were obtained from measurements lasting about 18 min The diagnostic accuracy of these five evaluation parameters were compared in order to investigate whether the method could be simplified without reducing diagnostic accuracy It was found that the pathologic cases in groups B, C and D were not clearly separated from the normal range by the evaluation parameters "Increase 9¼–16¾ min" (Fig 3) and this parameter was regarded as less valuable and therefore excluded The normal ranges of the parameters "Increase 1¾–16¾ min" and "Difference 1¾–16¾ min" were wider than the normal ranges of the parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" (Figs 2 and 4–6), probably because of some disturbing, irrelevant variation which becomes more important with time, for instance varying salivary flow This possibly explains why some pathologic cases not were separated from the normal range as effectively by the parameters "Increase 1¾–16¾ min" and "Difference 1¾–16¾ min" as by the "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" (Figs 2 and 4–6)

In accordance, Figs 7–8 demonstrate that the use of the two parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" (Fig 7) offered a better separation of patients with chronic inflammatory lesions of the parotid glands (groups B, C and D) from the control group (A) then the use of the two para-

meters "Increase 1¾–16¾ min" and "Difference 1¾–16¾ min"

Hypothetically, a combination of all these four evaluation parameters might offer better diagnostic accuracy then the parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min" alone, because of added information inherent in the parameters "Increase 1¾–16¾ min" and "Difference 1¾–16¾ min" However, this could not be demonstrated (Fig 9) and it was concluded that in clinical praxis the radiosialometric method can be simplified by using only the parameters "Increase 1¾–9¼ min" and "Difference 1¾–9¼ min", hence reducing the examination time from 18 to 10 min

Diagnostic accuracy

Figs 2, 5 and 7 demonstrate that the $^{99}Tc^{m}O_4$ accumulation process, evaluated in this way, offers good possibilities of separating glands with irradiation damage, collagen sialosis, chronic recurrent parotitis from normal glands This accords well with results obtained from scintigraphy and gammacameras (Shall & DiChiro, 1972)

It was found that bilaterally equal irradiation age caused a significant and symmetric ction of the $^{99}Tc^{m}O_4$ accumulation capac (Figs 2–6) but unilateral irradiation dam caused definitely abnormal side differences between the right and left glands capacities to accumulate $^{99}Tc^{m}O_4$ (Figs 2–6) The four patients with collagen sialosis had also bilaterally reductions of the $^{99}Tc^{m}O_4$ accumulation without side difference hence illustrating the generic character of this disease The five patients with chronic recurrent parotitis had less significant reductions (Fig 2) and one case had a definitely abnormal side difference (Fig 5), possibly illustrating a less generic character of this disease

Glands harbouring neoplastic processes were not separated from normal glands by this method with the exception of a small number of patients, in particular one patient with a low differentiated cancer, invading and destroying a large part of the affected gland (Figs 1, 2 and 5) Tumours of the parotid glands have been described as detectable by scintigraphy in some cases because of nonuniform distribution of the radionuclide in the affected gland (Shall & DiChiro, 1972) Warthin's tumour (papillary cystadenolymphoma) has been described to have an increased accumulation of $^{99}Tc^{m}O_4$ (Shall & DiChiro, 1972) but in the present study there was no side difference in the accumulation of the radionuclide in the two cases of Warthin's tumour However, tumours less than about 1½–2 centimeters, are difficult to demonstrate with scintigraphy (Shall & DiChiro, 1972) and tumours of this size or larger in most patients are easily revealed by palpation and fine needle aspiration biopsy is preferable in order to obtain a preoperative diagnose (Eneroth, 1973)

However, it is sometimes of interest to obtain information concerning the degree of parotid parenchyma destruction, caused by tumours in the region Such information can be obtained both by scintigraphy and radiosialometry The localization of the destruction might be demonstrable by scintigraphy but radiosialometry offers side difference as a quantitative evaluation parameter with high detectability

CONCLUSIONS

Radiosialometry can be simplified by the use of two oppositely placed detectors measuring the parotid accumulation of $^{99}Tc^{m}O_4$ during only 10 min after fast intravenous injection of the radionuclide

Side difference is a valuable parameter which cannot be sacrificed without a reduction in the diagnostic accuracy

The diagnostic accuracy of radiosialometry in generic lesions of the parotid parenchyma is high Thus all 14 cases with irradiation damage, collagen sialosis or chronic recurrent parotitis presented quantitative examination values objectively classified as definitely or probably abnormal whereas 49 of 50 control cases without parotid gland disorders pre

sented quantitive examination values objectively classified as definitely or probably normal

Irradiation damage collagen sialosis, chronic recurrent parotitis cause disturbances of the parotid gland cell metabolism and hence a reduced capacity of the parotid glands to accumulate $^{99}Tc^mO_4$

Collagen sialosis caused a bilaterally reduced capacity of the parotid glands to accumulate $^{99}Tc^mO_4$ without side difference

Neoplasm not causing extensive destruction of the parotid gland parenchyma could not be diagnosed with the radiosialometric method used

ZUSAMMENFASSUNG

Der zeitliche Verlauf der $^{99}Tc^mO_4$ Anreicherung in der Parotisdruse nach schneller intravenoser Injektion des Radionuklids wurde quantitativ radiosialometrisch bei 32 Patienten mit entzundlichen oder neoplastischen Erkrankungen der Parotisdrusen gemessen Die diagnostische Sicherheit von funf verschiedenen Auswertungsparametern wurde verglichen Wie es sich zeigte konnte die Untersuchungsmethode ohne Beeintrachtigung der diagnostischen Sicherheit dadurch vereinfacht werden dass drei der funf Auswertungsparameter nicht berucksichtigt wurden Dies bedeutete eine Verkurzung der Messzeit von 18 auf 10 Minuten Die diagnostische Sicherheit der Radiosialometrie war in 14 Fallen mit allgemeiner Affektion des Parotisparenchyms (Bestrahlungsschadigung Collagen Sialose und chronisch rekurrente Parotitis) hoch Alle 14 Falle zeigten abnorme radiosialometrische Werte Parotistumoren die keine ausgedehnte Destruktion des Parotisparenchyms verursachten wurden mit dieser Methode nicht entdeckt

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THE USE OF QUICK HARDENING POLYMERS IN THE SURGICAL TREATMENT OF CONGENITAL NECK FISTULAE AND CYSTS

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In 1970 the use of quick hardening polymers was decided on in the surgical treatment of congenital neck fistulae. The only correct method of treatment is a surgical one. Complete extirpation is a precondition for complete clinical success. The operation may be unsuccessful if the surgeon leaves a small fragment of fistula or of its lumen lining membrane, as this may cause a recurrence of the fistula.

A thorough study of the whole course of the fistula during the operation is therefore necessary. It often happens that a fistula is difficult to separate because of the varying diameter within its course and recesses or lateral branches. Complete lateral neck fistulae are difficult to separate particularly in their pharyngeal segment. Introduction of a metal or elastic probe into the lumen is often limited because of the winding course or stenosis of the fistula.

Since 1974 the same method with quick hardening polymers has been used during the extirpation of cysts of the neck.

METHOD

In the surgical treatment of congenital neck fistulae an X ray contrast examination performed before the operation permits one to recognize the volume of the fistula, as it is necessary to know how much of the liquid plastic mass should be prepared to fill up the whole fistula. In the first stage of extirpation a short fragment of the fistula with its external skin orifice is separated. A suitably thick needle with a small ball at its end is introduced into the lumen for about 2 cm, and the needle is then fixed above the ball by means of a silk ligature, thus becoming fixed in the outer orifice of the fistula. Thereafter the quick hardening liquid mass, prepared *ex tempore*, is introduced into the fistula lumen with a syringe. In lateral complete fistulae of the neck the injecting must be stopped at the moment the injected mass appears at the internal pharyngeal orifice of the fistula in the region of the posterior tonsillar arch. When the lateral fistula is not complete it has only its external orifice and canal, but no pharyngeal orifice. In this case injection must be stopped when the fistula is filled completely, which is manifested by resistance during the injection. The mass introduced under certain pressure fills up the lumen of the fistula with its probable branches or dilatations and after 3 to 4 minutes becomes hard enough to enable palpation of the course of the fistula. The blue polymers now in use offer a better visual contrast than the pink ones used during the first operations.

In the extirpation of congenital neck cysts the same quick hardening polymers have been used. After skin anaesthesia with 1% novocain a thick needle is introduced into the cyst. The cystic content is sucked out and measured

Fig 1 Complete fistula media filled up with contrast medium Narrow fistula with two dilatations one above the other

to establish how much of the liquid plastic should be prepared The same amount of liquid plastic mass is then introduced into the lumen of the cyst Three to four minutes later, after the mass has hardened the separation of the cyst can be started without any risk of rupturing its wall

Fig 2 Surgical specimen of the complete fistula media (from Fig 1) operatively removed (*right*) Polymer cast of the fistula lumen after the dissection of surgical specimen (*left*)

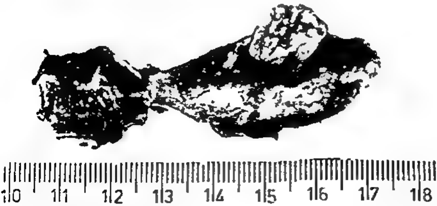

Fig 3 Surgical specimen of the fistula media filled up with polymer inflated along its full length from the hyoid bone (*left*) to its inferior end below the external orifice on the neck (*right*)

DISCUSSION

The method presented is simple and without danger to the patient When properly used, it guarantees filling of all the recesses and branches of the fistular system (Figs 1, 2) The hardening mass makes the separation easier and quicker, not only under visual control but also in tactile contact with the structure to be removed

The liquid polymers, introduced with a syringe, inflate the whole fistula lumen as well (Fig 3) This method of filling complete lateral neck fistulae does not require tonsillectomy in all cases The filling of the fistula facilitates the location of its pharyngeal orifice This method may also be used in extirpation of other congenital fistulae on the face (preauricular and others)

At e[illegible] paration is easier when they are filled The blue colour of the plastic mass inside the cyst contrasts against the surrounding tissues Puncture before filling of the cyst is of diagnostic value as it permits recognition of the cystic content

We have so far used the method presented above for the surgical treatment of two cysts of the neck and ten congenital neck fistulae

ZUSAMMENFASSUNG

Es wurde eine Methode der Fullung von angeborenen Halsfisteln mit einem schnell hart werdender Polymer wahrend der Eingriffszeit beschrieben Das gleiche Polymer wurde zur operativen Entfernung von Halszysten angewandt

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DE LA RÉUNION SCIENTIFIQUE DU

COLLEGIUM OTO-RHINO-LARYNGOLOGICUM

AMICITIAE SACRUM

DUBROVNIK, LE 15–19 SEPTEMBRE, 1975

Almqvist & Wiksell, Uppsala 1976

PRESIDENT'S ADDRESS

Mesdames et Messieurs
Sehr geehrte Damen und Herren
Ladies and Gentlemen

It is a particular pleasure to welcome you to Dubrovnik, this physically small but historically rich and important city—the city that possesses a rich heritage from the periods when historians called it the "Athens of the Slavs" This city, like the Greek "polis", proved that size was less important than freedom if one was to contribute to the welfare of mankind Over a thousand years of its free existence the Republic of Dubrovnik contributed more, in relative terms, to the advancement of human knowledge than many big states It gave birth to great scholars and artists such as the philosopher and mathematician Rugjer Bošković, the Roman professor of anatomy and theoretical medicine Gjorgje Baglivi, mathematician Ghetaldus, the great poet Gjivo Frano Gundulić, playwright Marin Držić, and others Of course, the Republic would not have been able to play such a vital part in the development of science and culture in the Balkans if it had not been for its economic strength which rested on the Ragusan merchant navy Ragusan ships were most numerous in the sixteenth and seventeenth centuries, when they linked western Europe and the Balkan peninsula and made Dubrovnik a major maritime trading centre The Ragusan fleet sailing under the sign of St Blaise, was a very serious competitor of the Venetian fleet sailing under the sign of St Mark For centuries, Dubrovnik was a mediator between

the Christian West and the Turkish part of the Balkans At the beginning of the nineteenth century both political and economic developments conspired to weaken Dubrovnik s economic significance just as they weakened the position of Venice too When the French troops headed by Napoleon s generals entered the area both Venice and Dubrovnik lost their political independence

At this happy moment when we open our ORLAS Congress here in Dubrovnik we should perhaps remember that we are meeting in a city whose patron saint was St Blaise And St Blaise was not only Dubrovnik s patron saint but also a protector and healer of people s throats If we wish to understand why so many saints were celebrated as protectors against various diseases during the Middle Ages we must look at the living conditions and mentality of the people of that time Man was at that time still largely helpless in the face of various elementary forces and was unable to understand their meaning Science was too undeveloped to supply answers to people s questions It is understandable therefore that natural phenomena were interpreted as manifestations of divine or infernal forces During the late Middle Ages when catastrophic idemics of pestilence leprosy diphtheria id fever and similar diseases took their in uncountable lives it was almost natural people should seek help in religion and miraculous powers of the saints seeing that medicine could neither heal them nor relieve their suffering Though lacking historical truth numerous legends circulating throughout Europe since the thirteenth century contained much beauty and poetry which stimulated the artists of the Renaissance to produce some immortal examples of art To the people who listened to them these legends brought at least some comfort and hope Now comfort and hope are the two psychological ingredients of great therapeutic importance which modern medicine has only recently begun to appreciate

Being a maritime country the Republic of Dubrovnik was certainly not immune against widespread epidemics of diseases and it was in view of this fact that St Blaise was chosen as its patron saint But at the same time a great deal of attention was paid to medicine too and material sacrifices were readily made to provide for health A detailed study of the appearance of the cult of St Blaise was made by J D Rolleston from London who reported his findings *to the Sixth International Congress on* Medical History held in Amsterdam and Leiden in 1927

The putting of crossed candles under people s throats on St Blaise s day (3 February) is a very old custom spread throughout the Christian world Naturally the practice was particularly strong in the Republic of Dubrovnik whose patron saint St Blaise became as early as 1190

The belief in the supernatural powers of healing and the mistrust of mediaeval official medicine which consisted mainly in the uncritical adoption and often wrong interpretation of classical medical principles remained the characteristic feature of western medicine for many centuries until the arrival of the Renaissance But it should be stressed that even during these centuries there were some fine examples of man s successful struggle against infectious diseases In this respect Dubrovnik occupies a very distinguished place in the history of medicine of that period Medical problems were often on the agenda of the city s Council of the State and Grand Council The authorities paid a great deal of attention to public hygiene one of the major events was the decree of July 27 1377 instituting quarantine against contagious diseases Dubrovnik s position was such that it could not easily escape epidemics of plague It is estimated that some 25 000 of the Republic s inhabitants died of plague between 1348 and 1374 From the recorded list of symptoms we conclude that it was the lung pestilence Looking for ways to protect the city the authorities remembered their experience with the isolation of lepers and the Grand Council decided

(on July 27 1377) by 34 votes against 13 that no citizen of Dubrovnik or foreigner coming from infected parts of the world could enter the city or its surroundings unless he first underwent cleaning on the island of Mrkan or in the nearby village of Cavtat The local people were not allowed to visit these seamen without special permits issued by the sanitary authorities Other Mediterranean ports soon followed Dubrovnik s example Special officers were appointed to supervise the implementation of the measure and to punish those who disobeyed it · Sanitary officers checked the borders very carefully particularly from the sea and scrutinized the medical certificates of those arriving in the Republic No cargo was allowed to be unloaded before it was carefully checked The sanitary officers who enforced the regulations reported directly to the Rector and the Council of the State Fines for those who violated the regulations were severe a person escaping from the quarantine was fined between 50 and 100 perpers which was then an enormous sum of money Graver offences or those committed by the citizens of Dubrovnik were punishable by branding the person in question with hot iron or by having one of his ears cut off In 1628 Dubrovnik built a large and modern lazaretto which was long regarded as the best quarantine in the world

The small Republic of Dubrovnik as we see had a well organized public hygiene service already in the fourteenth century It is no mere chance that the figure of Asclepius stands on the finest capital in the Rector s Palace here in Dubrovnik But knowing the orientation of the people in old Dubrovnik one concludes that it was economic reasons that prompted them to institute a very strict sanitary rule They knew very well that a healthy population was a country s most valuable asset It is important to note in this connection that the first pharmacy was opened in the Franciscan monastery in Dubrovnik in 1317 that the municipal authorities opened a public pharmacy in 1420 and that the first orphanage was built in 1432 The Republic also established hospitals and old people s homes The first excellently organized hospital named Domus Christi was opened in 1540 The Dubrovnik Archives still keep the Hospital Rules from that hospital with very clearly specified duties for doctors and nurses

Scientific medicine began to develop in Dubrovnik at an early date thanks to the city s close connections with Salerno which started a new trend in medicine Prominent physicians from different Mediterranean countries served in Dubrovnik leaving behind visible traces not only in the form of new medical ideas applied in practice but also in written works Shortly before the fall of the Republic Dr Luka Stulli introduced vaccination against cowpox and wrote a treatise about plague and anthrax Dr Stulli also started vaccination against smallpox according to the method proposed by Edward Jenner three years earlier He obtained the vaccine from his friend Luigi Aloysio Careno in Vienna In 1805 Stulli published a booklet about vaccination He was the first physician in Dubrovnik to write medical texts in Croatian was interested in popular medicine and tried to improve hygienic standards among the people

Several citizens of Dubrovnik established their medical reputations abroad Thus for instance Dominko of Dubrovnik was a professor at the Universities of Bologna and Siena towards the end of the Middle Ages Some of his commentaries on Arab medical texts and original discussions of podagra calculosis poisons and medicines have been preserved in manuscript form The manuscript of an important study on vegetable animal and mineral medicines written by the Dubrovnik physician and philosopher Grgur Budislavic (early 16th century) is now kept in Bologna The best known of the Dubrovnik physicians was undoubtedly Giorgio Baglivi (1668–1707) who became a famous professor of anatomy and theoretical medicine in Rome His writings were used for a long time by all the scientists of the physical orientation in medicine

His experiments, particularly those involving injections in the blood circulation system and spine of animals, were very important for the development of pharmacology It is interesting to note that Baglivi provided theoretically for the possibility of specific chemotherapy In many ways, the place of Baglivi in the de velopment of modern medicine can be com pared to that of Rugjer Boškovic in the development of modern physics Over two hundred years ago, Boškovic, a philosopher and professor at various European universities, formulated a view of the structure of matter which foreshadowed in an interesting way modern developments in physics and theory of relativity

In the not too distant past, Dubrovnik led the way in the Balkans in the introduction of new methods in medicine, particularly in surgery On April 20, 1847, Frane Lopišic operated a woman's breast cancer in the Dubrovnik Hospital using ether as an anesthetic given through a mask designed by his colleague Pinelli With the same mask, Pinelli experimented with the use of ether on himself In this operation one whole breast was excised If one remembers that the first operation under ether in the world was performed by John Col- ns Warren (1778–1856) in Boston on Octo- r 16, 1846, then the Pinelli Lopišic ether anesthesia must have been one of the first such attempts in Europe and especially in the Bal kan area

Medical science and particularly university education in medicine, is not neglected in modern Dubrovnik either We are proud to have here in Dubrovnik the Inter-University Centre for Postgraduate Studies (opened in 1972), in the work of which scientists, scholars and students from over 40 countries have participated so far The Centre operates under the auspices of the University of Zagreb and includes among its members many of the members of the International Association of Universities Students and teachers from non-member universities can also participate In the course of 1974/75 academic year, im portant problems of philosophy and humanism were discussed, the two disciplines that the Republic of Dubrovnik never ceased to culti vate The topics included Philosophy and Sci ence of Humanism, Theory of Knowledge, European Security and Cooperation, Practice and Theoretical Perspective, Man and Envi ronment, New and Old Materialism in the Philosophy of Rugjer Boskovic, Resource Energy and Multinational Cooperation Such facts show that this small city of Dubrovnik will continue to play a role in the development of world science in the future To all scientists and scholars it will open its beauty, harmony and hospitality in the interest of scientific progress in the world These are, in short the reasons which have prompted us to organize this meeting here I wish you a most cordial welcome and hope that you will meet new friends and come again to enjoy the shade of the Dubrovnik walls and the azure beauty of the Adriatic These ancient walls which pro tect the old city with its magnificent palaces quiet churches and narrow streets will remind you of the freedom and independence of this small Republic which has produced such great figures in science and art

I Padovan

VICE PRESIDENT'S ADDRESS

Ladies and Gentlemen
Members of the Collegium

It is a great pleasure and honour for me to greet this distinguished gathering in the name of the Organizing Committee and the Zagreb Faculty of Medicine After twenty years our country is once again the host of this eminent scientific reunion in this traditional centre of culture and freedom in our Dubrovnik

The Zagreb Faculty of Medicine the oldest among the Southern Slavs is thus for the second time your host Although it was founded only in 1917 the efforts towards its foundation began already at the end of the 18th century and continued uninterrupted to the final achievement This fact need not surprise us when we remember that the Turkish border was fifty kilometres away from Zagreb and that the age long struggle between East and West was waged on our territory The Foundation of this Faculty was made more difficult owing to the lack of economic prosperity which is always a condition of every scientific development including medicine But in this arduous past there were also radiant moments and examples which bear witness to our capabilities in the field of medicine and were actually forerunners of the development of health service in our country This applies primarily to Dubrovnik and to our other coastal cities which were in constant good relations with the well known centres of medicine at Salerno Bologna Florence and Padua Several of our physicians distinguished themselves by considerable knowledge and medical skill so as to become professors at these eminent centres of learning and of the art of medicine So for example our first woman physician Draga Slava is mentioned at Venice in the 14th century Also in the 14th century a certain Domenico of Dubrovnik becomes professor of astrology and theoretical medicine at Bologna Matija Vlasic is made junior professor at Rostock in the 17th century Djuro Baglivi is elected professor of anatomy and later also of theoretical medicine in Roma As an important commercial centre on the Mediterranean Dubrovnik organized on the territory of its Republic a model health service for those days perhaps one of the best and most modern in the whole of Europe in connection with this a modern hospital was founded there in 1540 in which curative medicine seems to have played the most important part

The beginnings of higher education in Croatia date from the opening of a Jesuit Academy in Zagreb which in 1669 received a charter from king Leopold I giving it all university privileges In 1776 the same Academy was proclaimed a Royal Academy of Sciences with three faculties (of theology, philosophy and law) It was then that the first initiative was taken to found also a faculty of medicine but instead of a chair of surgery the Academy of Zagreb was given a chair of Hungarian language and literature However during this period some schools of medicine came into being in our country So in 1806 two medical schools were founded in Dalmatia and in 1812 an attempt was made to open a surgical school in Zagreb All these efforts just as in later periods always met with opposition on the part of the foreigner even when in 1874 a university having unlimited rights was officially founded in Zagreb In the meantime owing to the ever increasing number of doctors to the founding of the Corporation of Physicians in 1874 as well as to bad health

conditions in large areas of the country, it became imperative that a faculty of medicine should be created Even means had to be collected from among the notable and well-to-do citizens until finally in 1917 the more than a century old dream could come true and the first faculty of medicine in the Balkan Peninsula founded

It is interesting to note that among the first three appointed professors was the Nestor of our otorhinolaryngology, Professor Mašek, who on that occasion said "Our Faculty of Medicine will not be a nursery only for us Croats but also for all the Slavs of the South as a symbol of their cultural and national unity" And so otorhinolaryngology was introduced in 1919 in the curriculum and became a compulsory subject of clinical instruction Consequently also in our situation otorhinolaryngology attained its proper place almost at the same time as in many first-rate medical centres of Europe This discipline has thus played an important role in Yugoslavia itself, setting an example for all future faculties of medicine in the country, and by its professional and scholarly work it gained a good reputation even abroad Šercer was one of the first members of the Collegium, and he was later joined by ušic and Podvinec They together formed the d Zagreb school which helped to develop orhinolaryngology in this country and train new members of the Collegium so that in the past few years Yugoslavia has been able to raise its full quota of members for this distinguished society.

I think that the otorhinolaryngologists at the Zagreb Faculty of Medicine have not only developed their branch of study but have made a palpable contribution to medical thought, education and scientific development in general both among their staffs and in a wider circle I am of the opinion that after Zagreb and Belgrade in 1955 we could hardly have chosen a more suitable place than Dubrovnik for this gathering of the most prominent otorhinolaryngologists of the world When four centuries ago (in 1579) Dominko Zlataric was made Rector Artistarum at the University of Padua, Professor Galluci delivered a solemn speech in which he brought out all the good qualities of the newly elected Rector and in the first place he mentioned that Zlataric was born in Dubrovnik—*in nobilissima totius orbis terrarum Republica quae ceteris Rebus publicis antecellit*

On behalf of the Organizers and the Zagreb Faculty of Medicine I wish you all a very pleasant sojourn here May the historical monuments of this city remind you of the exceptional accomplishments of this nation which even in the teeth of fearful odds, fighting against all possible invaders, was able not only to preserve its national entity but also to foster medical thought and things of the spirit

Z Krajina

Acta Otolaryngol 81 161–166, 1976

RETRACTION OF THE DELTO PECTORAL FLAP

Clinical Observations and the Design of an Experimental Model

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Abstract The human delto pectoral flap retracts markedly across its breadth but remains the same length after elevation This unidirectional retraction declines with age Flaps in a similar position on the pig running in the same direction behave in a similar fashion suggesting that such flaps are a suitable animal model for the study of this property

The delto pectoral flap was first described by Aymard in 1917 for reconstruction of the nose It was forgotten until Bakamjian revived it in 1965 for reconstruction of the pharynx after total pharyngo laryngectomy since then it has rapidly gained in popularity and is now used for reconstruction of many defects in the head and neck particularly the skin of the neck within the oral cavity, and the oropharynx in addition to pharyngeal replacement as described by Bakamjian

In an article on the use of the delto pectoral flap McGregor *discussed the fact that the lower border of the flap has more slack than* its upper border due to the presence of extensile skin in the anterior axillary fold, this gives the flap greater length when it is turned upward since it is the lower border which is under greatest tension when the flap is turned upwards McGregor illustrated this by a photograph of a delto pectoral flap drawn on the skin of the chest when the arm was abducted from the side the pattern of the flap elongated furthermore when the flap was actually raised it similarly elongated and retracted from side to side (McGregor & Jackson 1970)

The presence of excess skin in the axilla which must be present to allow the arm to be abducted from the side could explain the elongation along the long axis but would not explain the diminution which observation shows takes place in the vertical height of the flap and this change is more likely to be due to the inherent tension of the skin Thus the delto pectoral flap remains approximately the same length and may even elongate but retracts across its width after it has been raised

This observation raises several questions Do all long rectangular flaps retract in this way or is the retraction of the delto pectoral flap dictated by lines of tension running in one direction only if there are unilateral lines of tension what is their cause, and is it necessary to take the retraction of a flap into account *when planning a flap?*

The investigation into some of these questions will be reported elsewhere (Stell & Green 1976) this article is concerned with measuring the changes which take place in human delto pectoral flaps and with the design of an experimental model on the pig to study this

MATERIAL AND METHOD

I *Human delto-pectoral flaps*

Delto pectoral flaps, for use in reconstruction after excision of a head and neck cancer, were

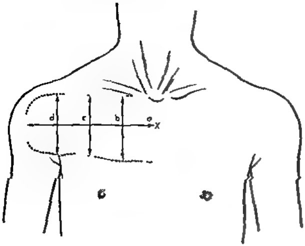

Fig 1 Diagram of a delto pectoral flap with points to be measured marked

marked out on 24 patients The flaps were marked with methylene blue they extended laterally to the acromion the superior border lay over the clavicle and the upper four perforating branches of the internal mammary artery were included in the base of the flap The flap was measured along its length (i e medio laterally) from a point marked on the midline to the extreme end of the flap (length *a* in Fig 1) and across its width (i e supero inferiorly) one quarter one half and three quarters of the way along the flap (lengths *b* *c* and *d* in Fig 1) The flap was then elevated in the usual way including the pectoral fascia on the flap stopping elevation medially at the medial end of the intercostal space to preserve the perforating branches of the internal mammary artery The flap was then replaced and measured at the same places as before A typical flap is shown after elongation (Fig 2)

The measurement of the width of the flap after elevation for the lengths marked *b* and *d* on Fig 1 were discarded because the line *b* was too near the origin of the flap to allow the elastic recoil to exert its maximal effect and because *d* was placed at the axilla and

Fig 2 A delto pectoral flap after retraction

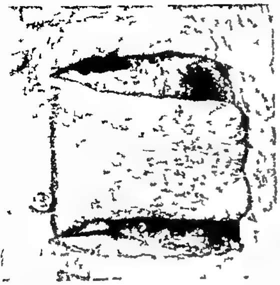

Fig 3 A pig flap after retraction

accurate measurements at this point were difficult because of excess tissue present at the site and because of the curvature of the skin The results for change of length (measurement *a*) and change across the flap (measurement *c*) were then analysed The mean and standard error of the changes in size of the flap along its length and across its width at its centre were calculated and the difference between these two was analysed by a Students *t* test for unpaired data

The retraction across the flap was then broken down into two groups for men and women and the data analysed by a Students *t* test for unpaired data This analysis was done because it has been suggested that the tension in the skin of this part of the chest differs between men and women because of the presence of breast tissue (Kraissl 1951) The retraction across the flap with respect to age was analysed by the method of least squares to show any correlation between retraction and age

2 *Coronal abdominal flaps in the pig*

In order to answer many of the questions raised by predominantly unilateral retraction an animal model is necessary The pig was used since it has been accepted by previous workers (e g Patterson 1968) as the animal with skin which most closely resembles the human Flaps were studied as near as possible in position to the human delto pectoral flap (i e based on the ventral surface of the trunk) based medially and running parallel to the coronal axis

Ten pigs of a Large White/Landrace cross were used in the 30–35 kg range Anaesthesia was induced with halothane and continued with halothane nitrous oxide and oxygen

Thirty nine coronally placed flaps based medially were marked out on the pig s abdomen They ranged in size from 100×50 mm to 50×20 mm (length to base) and their position and the side of the abdomen on which they were placed was randomised (these flaps were also used in an experiment to compare the viability of rectangular against triangular flaps—hence their randomisation as to side) The flaps were then elevated and measured along and across the flap at the centre of the sides The flaps were finally sewn back in place for studies on viability A typical flap is shown (Fig 3)

The raw data were analysed to find the mean and standard error of the percentage change in size along and across the flaps and the differences between the change in length against the change in width for each flap was compared by a Students *t* test for unpaired data

RESULTS

1 *Human delto pectoral flaps*

The complete results show that the change in length of the delto pectoral flap ranged from an increase of almost 7% to a decrease of 17%

Table I *Retraction along and across human delto-pectoral flaps*

	Along the flap	Across the flap
Mean	1 6	20 1%
S E M	1 11	1 06

t=12 0 d f =46 p<0 001

Table II *Retraction across delto-pectoral flaps with respect to sex*

	Male	Female
Mean	20 3%	19 4%
S E	1 32	1 89

$t=0\,40$ d f $=22$ N S

but the overall mean was a slight retraction of 1 6% Thus for practical purposes the flaps stayed the same length As regards change of size across the flap at its centre, the flaps always retracted with a mean of approximately 20% (Table I) There was a highly significant difference in the change in shape along and across the flap

There was no significant difference in the retraction across the flap with respect to sex (Table II) The results of the analysis by the least squares method for the changes across the flaps with respect to age are shown in a graph (Fig 4) The correlation coefficient ($r=-0\,56$, $t=3\,21$, d f $=22$, $p<0\,005$) showed that there was a significant correlation between the change in shape and age, the correlation being negative so that the retraction de-ned with increasing age

Examination of the results for change in pe along the flap also suggested that there might be a correlation with age A graph (not shown here) did not show as clear-cut a picture as Fig 4 but breaking down the results into two groups above and below the age of 55 (Table III) did suggest a trend flaps above this age elongating after cutting, and flaps below this age shrinking along the flap The numbers were not large enough for analysis by a χ^2-test

2 *Coronal abdominal flaps in the pig*

Two thirds of the flaps elongated after they had been elevated and one third retracted along their long axis the net result was an increase in the mean length after elevation, whereas the flaps all retracted markedly (about 40%) from side to side The mean, standard error and t test of the changes along and across the flaps are shown in Table IV The differences were statistically highly significant

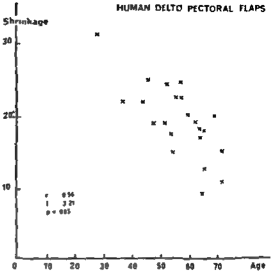

Fig. 4

DISCUSSION

These results indicate that both abdominal flaps parallel to the coronal axis of the pig and the human delto pectoral flap undergo a change in shape after they have been elevated shrinking across the flap but remaining more or less the same length This change in shape is thus unidirectional and in the same direction in both human and pig flaps, so that coronal flaps on the abdomen of the pig appear to be a reasonable animal model for studying retraction In the human the change is more pronounced in younger patients suggesting that it is mediated by elastic tissue

Table III *Human delto-pectoral flaps*

	Across the flap	
Age	Shortened after cutting	Lengthened after cutting
55 and under	8	1
Over 55 years	5	10

Table IV *Change of shape of rectangular flaps parallel to the coronal axis (pig)*

	Along the flap	Across the flap
Mean	+7 0%	−40 6%
S E M	2 0	0 8

t 21 9 d f 76 $p<0\ 001$

McGregor s observation (1970) that the flap elongates after it has been cut was only an incidental observation in a long article about the use of this flap and it was not investigated further by detailed measurements The evidence presented here supports the contention that some flaps do indeed elongate after they have been cut, particularly in the elderly, but that in a group of moderate size there is no overall mean change for practical purposes in the length of the flap The clinical significance of this change of shape has already been discussed by McGregor—it is obvious that a flap which retains its length rather than shrinking has clinical advantages since it has less distance to travel and can be cut shorter than if shrinkage needed to be allowed for This aspect does not need to be developed further here therefore

The results showed that the delto pectoral flaps always shrank across their long axis in patients under 55 years old the flaps became slightly shorter along their length and over this age they tended to become slightly longer after elevation It thus appears that the tension operates maximally in a vertical direction With the well known decreasing elasticity which occurs with age the effect of the tension along the flap appears to disappear after the age of 55 it is postulated that the flap then behaves like a tube of toothpaste—pressure from the sides pushes tissue outwards at the end

The observation raises several questions which have been investigated further Do all long rectangular flaps behave in this way or is the change in shape dictated by a unidirectional tension in the skin? Is the retraction of a skin flap dictated by the length to breadth ratio of the flap and what is its relation to the original size of the flap? Finally, if there is a unidirectional tension in the skin, what causes it? The standard description of elastic tissue (Ham, 1974, Pinkus & Mehregan, 1969) states that the elastic tissue of the dermis is arranged haphazardly such an arrangement would not explain unidirectional tension The results of investigation into some of these aspects are in publication elsewhere (Stell & Green 1976)

ACKNOWLEDGEMENTS

The author is grateful to the following Professor Ivo Padovan President Collegium O R L A S for the invitation to read this paper to the Research Committee of the Liverpool Area Health Authority (Teaching) for making funds available to Professor R Shields Department of Surgery University of Liverpool for advice and for making facilities available and to Miss P Hughes for preparing the manuscript

RESUME

Il est bien connu qu un lambeau se retracte apres sa decoupe La retraction peut cependant avoir une polarite directionelle si bien que le lambeau change de forme dans une seule direction C est le cas avec le lambeau delto pectoral qui se retracte lateralement mais reste de la meme longueur apres son elevation Un protocol experimental a ete effectue pour illustrer cet effet directionnel de la retraction elastique ses bases histologiques et sa signification pratique pour la viabilite d un lambeau cutane

ZUSAMMENFASSUNG

Es ist wohl bekannt daß ein Hautlappen schrumpft nachdem er geschnitten wurde Dieses Schrumpfen kann aber eine die Richtung beeinflussende Eigenschaft haben so daß der Lappen seine Form nur in einer Richtung andert Das ist der Fall mit dem delto pectoralen Hautlappen der nach Abhebung von Seite zur Seite schrumpft aber dieselbe Lange behalt Als Beweis werden experimentelle Resultate vorgelegt um sowohl den Richtungseffekt des elastischen Schrumpfens als auch die histologische Grundlage unter die praktische Bedeutung für die Lebensfähigkeit des Hautlappens zu illustrieren

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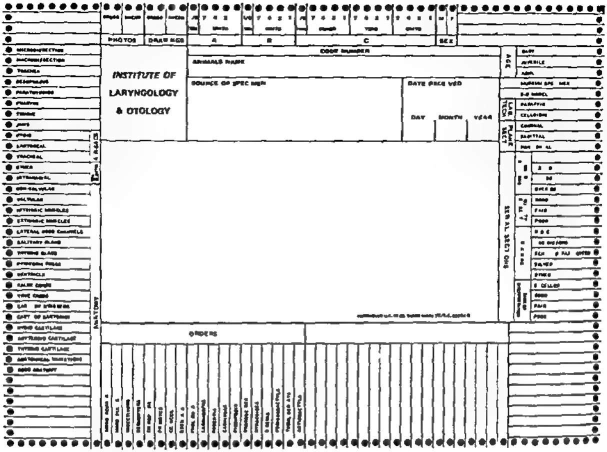

Fig 1 Custom-designed punch card for recording relevant information after serial sectioning

Much of our animal material has come from ological Gardens being removed at post tem either by the Veterinary Pathologist or the good services of an interested laryngologist Immediate fixation in formal saline has allowed storage until, in the case of specimens obtained abroad, I can collect the material personally Importation of preserved larynges

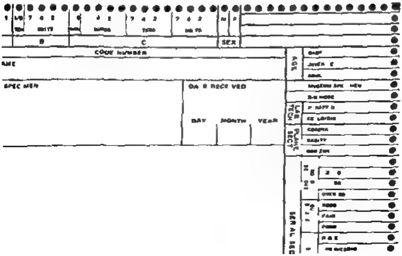

Fig 2 Close up of a portion of punch card

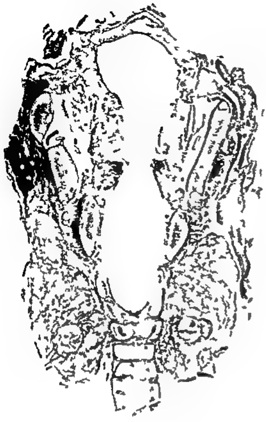

Fig 3 Coronal section human larynx ×?

presents no problem in England and gives the Customs Officials something to talk about!

Expeditions by the Institute Zoologists has resulted in excellent material all fixed intra vitally and identified by experts Naturally this is confined at present to the smaller and more manageable mammals

Standard staining methods similar to those used in the human larynx are used though requiring some modifications in the case of unusually small or large specimens Two tech nicians are constantly employed on this pro ject in order to deal with the ever increasing volume of work

(*a*) Haematoxylin and Eosin remains the single most useful procedure allowing differen tial staining of cartilage and bone This is most useful in deciding the degree of ossification of the laryngeal cartilages Some such as the Chiroptera are always completely ossified since their unusually well developed intrinsic musculature obviously requires strong attach *ment*

Muscle fibres and thyroid colloid stain deep pink which permits ready identification

(*b*) Verhoeff's elastic stain combined with Van Gieson allows the differentiation of con nective tissue and is obviously of great value in detecting the various fascial sheets such as the cricovocal membrane Elastic fibres are black with connective tissue red the muscle stains a bright contrasting yellow

(*c*) Periodic acid Schiff (PAS) stains for

Fig 4 Coronal section of the cat larynx (*Felis domes ticus*) ×4

Fig 5 High power view of superior laryngeal nerve (cat) ×20

Fig 6 High power view of divisions of internal branch superior laryngeal nerve (cat) ×20

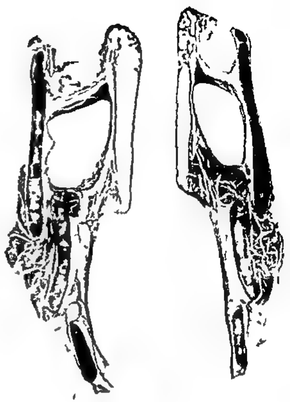

Fig 7 Coronal section larynx of the horse (*Equus caballus*) ×2 Note the internal branch of the superior laryngeal nerve

carbohydrate giving red or magenta for PAS positive substances with blue nuclei This technique is probably of more importance in the examination of pathological material but does differentiate polysaccharides (glycogen) mucoproteins and glycoproteins and therefore could provide useful information

(*d*) Glees Silver stain is only one of the many techniques employing a silver salt to stain nerve fibres With this it is possible to stain both large and small whole organ sections not easy even in skilled hands but of great importance in following the pathways of the laryngeal nerves Nerve cells and axons stain black and Figs 3 4 5 and 6 shows this method used in man and the cat Experimenters using the cat have commented on the unusually sensitive larynx which may result in fatal laryngeal spasm Our sections of *Felis domesticus* show that the superior laryngeal

Fig. 8. Coronal section horse larynx in an animal with an inherited left recurrent nerve paresis. ×1.75. Note atrophy of intrinsic muscles.

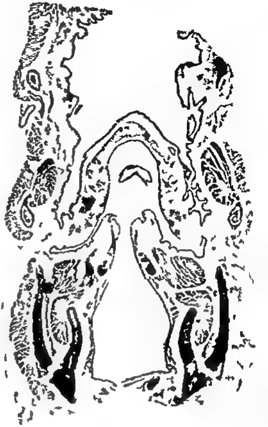

Fig. 10. Coronal section mongoose larynx (*herpestes*). ×6.

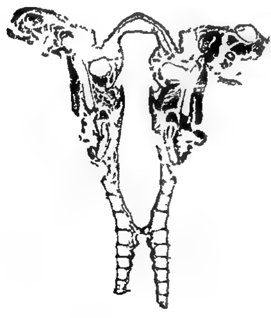

Fig. 9. Coronal section dog larynx (*Canis familiaris*). ×3.

nerve is unusually large and divides into a surprisingly large number of branches. Since this animal possesses no ary epiglottic fold it is possible that the sensory nerve supply is greater than the Horse (*Equus caballus*) where the high folds direct liquid food away from the glottis (Fig. 7). Similar staining methods are being used to study the left recurrent nerve paresis which is so common in thoroughbreds resulting in 'Roaring'. This is probably an inherited condition but no information is available as to whether the intrinsic branch of the superior laryngeal nerve is entirely sensory in this species. Indeed no study has been made of the nerve supply in this animal at all (Fig. 8).

Apart from the preparatory work of Negus we possess little in the way of three dimensional knowledge of the mammalian larynx

Many anatomical features such as the presence and relationships of laryngeal air sacs, nervous pathways and even the lining epithelium can only be obtained by whole organ sectioning The acquisition of such fundamental information will eventually allow an informed reappraisal of many theories and also provide a reference collection for all interested comparative laryngologists to study the intricacies of this exceptional organ (Figs 9, 10)

RÉSUMÉ

Cet article comprend les détails brefs d une étude tri dimensionelle a long terme de la fine structure du larynx mammifère en employant les coupes en série de l organe entier L emploi de colorant differentiel y compris la technique d'argent de Glees est essentiel pour bien profiter de l information Une carte perforée (de computeur) de dessein spécial rendra capable de retrouver rapidement l information petinente n importe quel investigateur de l avenir Il est donc pensé que les spécimens de tout larynges mammifères seront compris Cet article renferme des illustrations du chat du chien du cheval et du mangouste

ZUSAMMENFASSUNG

Die Arbeit behandelt Details einer langfristigen dreimensionalen Untersuchung der feinen Struktur des malen Kehlkopfes wobei ganze Organ Serienschnitte sind Unterschiedliche Farbstoffe einschliess Glee s Silber Methode sind notig um eine gute Kenntnis zu erlangen Eine speziell entworfene Lochkarte wird es kommenden Nachforschern leichter machen sehr schnell anwendbare Information herauszufinden Man erwartet dass Exemplare aller Saugetierkehlkopfe darin vorkommen werden Abbildungen von Katze Hund Pferd und Mungo sind in diesem Artikel einbegriffen

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England

DISCUSSION

I Friedmann You have pointed out the importance of perineural invasion of cancer in this organ Since such lesions may be irregularly distributed might not a more detailed knowledge of the distribution of the laryngeal nerves be of some assistance to the pathologist?

P M Stell Have you found any tumours in animal larynges and have you any comments to make about the laryngeal air sacs and laryngocochlea?

D Harrison (Reply) to *Mr Friedmann* It is hoped that our studies on the normal human larynx will provide detailed information of the pathways of the internal laryngeal nerve supply and its relationship to the fascial planes Translating this information to the larynx with cancer will then help us to answer the questions now posed

To Mr Stell As yet little reliable information is available as to animal air sacs since they can not always be seen on morphological examination Only three dimensional reconstructions will give this information but it seems likely that in most species the air sacs if present will not be entirely saccular

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STUDIES ON THE ANATOMY OF THE FACIAL NERVE

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Abstract The present study on the anatomy of the human facial nerve is based on the results of a series of gross dissections, and histological and electro-diagnostic examinations From our findings it follows that there is a definite spatial orientation of the peripheral branches in the facial nerve trunk The results of the morphologic studies are in agreement with those obtained by electric stimulation combined with electromyography

Problems of pathophysiology and surgery have repeatedly given impetus to detailed studies on the normal anatomy of the facial nerve The investigation of spatial orientation of the nerve fibres within the main trunk is of particular interest in the elaboration of diagnostic and surgical procedures in cases of facial nerve neurinoma, hemifacial spasm and blepharospasm

A review of literature in this field, however, reveals that there is no agreement with respect to the intraneural spatial organization of the nerve fibres and the existence of a cross-sectional arrangement of fibre bundles, innervating respective facial muscle groups, is still in question Sunderland & Cossar (1952) demonstrated that there is no consistent pattern of nerve fibre bundles within the facial nerve in man They found an intraneural plexus of nerve fibres, which revealed their destination only after they coursed from the stylomastoid foramen to their termination in the face Harris (1968) confirmed these findings by experiments in monkeys when partial sectioning of the proximal portion of the main trunk failed to produce a corresponding pattern of degeneration in the distal course of the facial nerve. Scoville's (1955) clinical observations also supported the idea of no spatial orientation, as he reported that partial sectioning of the proximal trunk of the facial nerve when treating patients with hemifacial spasm did not appear to affect one division more than the other

On the other hand, Hofmann (1924) described a well defined course of the Chorda tympani and of fibre bundles extending separately to the upper and lower regions of the face Miehlke (1958) added further support to the theory of an intraneural spatial organization of facial nerve fibres by means of intra-operative mechanical stimulation of the tympanomastoid segment Eynès & Chouard (1963) who reconstructed the topographic arrangement of the nerve fibres by the method of Born (1883) were able to prove a distinct spatial orientation of the facial nerve throughout its course from the brainstem to the periphery Observations by Pohlmann (1937), Miehlke (1973) and other authors, such as Saito et al (1970) and May (1973) also suggest the presence of a well defined cross-sectional pattern of facial nerve fibre bundles, innervating specific groups of facial muscles

In the present paper we have made the attempt to gain further information on the topographic (i e spatial) arrangement of facial nerve fibres in man by the following methods:

1 Gross dissection of the nerve under the surgical microscope in cadaver

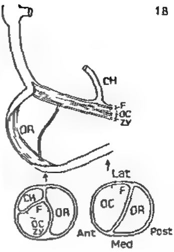
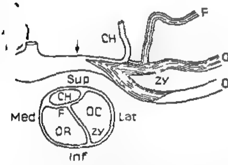


Fig. 1 A–B. At the level of the stylomastoid foramen the oral branch lies posterior to the main bundle; its volume amounting to one third of the nerve trunk. Isolated, it forms a crescent moon with an irregular concave profile in which the longitudinal impressions correspond to protuberances of the main bundle and vice versa. A parallel arrangement of fibres was observed in all specimens and the oral branch could be peeled away from the main trunk without interference of any crossing or intertwining fibres. The volume of the oral branch remains constant. At the level of the second bend it takes the shape of a half moon and occupying the postero-medial part of the nerve it lies closest to the horizontal semicircular canal. Within the labyrinthine segment the oral branch slides partially under the main trunk and takes a slightly rotating course until it is located postero-medially to the geniculate ganglion. In the meatal segment the oral branch courses in a postero-superior position. *CH*, Chorda tympani; *F*, frontal; *OC*, ocular; *ZY*, zygomatic; *OR*, oral branch.



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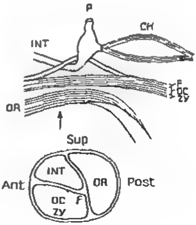

3

Figs. 2–3. At the level of the stylomastoid foramen the upper branch of the facial nerve containing temporofrontal and zygomatic fibres is located first in an anterior position. Adjacent to its lateral margin lies the Chorda tympani. At the level of the second bend and within the tympanic portion it is located supero-laterally. Thus at the level of the tympanic segment the Chorda tympani is located superiorly. *P*, petrosal nerve; *INT*, intermedius nerve.

At the level of the stylomastoid foramen the temporofrontal fibres are running antero laterally. In the upper two thirds of the vertical portion they lie central to the Chorda tympani at the margin between the oral and ocular branches. At the level of the geniculate ganglion the upper branch is located antero inferiorly, crossing obliquely with the fibres of the intermedius nerve. In most specimens ocular and zygomatic branches were not readily discernible from each other; the longitudinal dissection however showed a parallel course of the fibres and proved again the absence of an interfascicular crossing.

2 Histological examination
3 Electric stimulation of the tympanomastoid segment during surgery under electromyographic control

MATERIALS AND METHODS

Three facial nerves were carefully dissected and extracted from the temporal bones of human cadavers and, after fixation in osmium tetroxide, embedded in Epon and stained with toluidine for examination of crosswise sections

One nerve was used for longitudinal frozen sections which were stained with Sudan red

Four nerves were dissected in fresh human temporal bones without previous fixation six facial nerves were dissected, extracted and fixed *ad modum* Thomas (1955) after 24 hours' fixation in 4% formalin the nerve is washed in tap water and osmicated for 24 hours A treatment with a mixture of equal parts of water and glycerin for a further period of 24 hours renders the tissue sufficiently elastic for an accurate dissection under the surgical microscope It must be kept in mind, however that this method has its technical limits for the preparation of extremely fine and fragile nerve structures

RESULTS

Examination of transverse and longitudinal sections of the facial nerve does not reveal any conclusive facts about the course of the fibres By analysing the longitudinal sections we could only confirm the findings of Sunderland & Cossar (1952) whereby tracing a single fibre in a single section cannot give precise information about its course over a longer distance

Dissection of the nerve in fresh temporal bones encounters technical difficulties as post mortem changes tend to produce artefacts and increase the fragility of the fibres For these reasons we had to find a more appropriate method of dissection and therefore used the fixation procedure of Thomas (1955) Although dissection and complete extraction of the nerve from its canal as well as fixation may cause difficulties in spatial orientation of the fibre bundles in relation to neighbouring structures, dissection of nerve tissue becomes much easier and more precise Figures 1–4 reproduce the results of our anatomical studies, carried out under the control of the operating microscope

Finally we present the results obtained by intraoperative electric stimulation of the facial nerve We have used the following technique

After decompression and splitting of the nerve sheath in its tympanomastoid segment, the main trunk is divided by blunt dissection into three or four fascicles over a distance of 5–6 mm, proximal to the stylomastoid foramen The fibre groups are thus chosen at random, as no anatomical structure exists delimiting the fascicles within the nerve trunk Each of these fibre bundles is stimulated separately by supramaximal square-wave stimuli by means of a bipolar microforceps (6 volts, 1 Hz, 0 2 mSec) The response of the facial muscles is observed directly and simultaneously The summation potential is recorded by surface electrodes

We have carried out this experimental procedure in 2 patients one suffering from hemifacial spasm and one with blepharospasm and we were able to chart the following functional organization of fibres in the vertical segment (Fig 4)

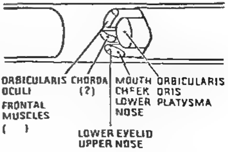

Fig 4 Map of fibre bundles in the vertical segment (surgeon s view right facial nerve)

This topographic arrangement, i e the spatial orientation of nerve fibres, is in full agreement with the results of the anatomic investigation

DISCUSSION

Analysis of the results of gross dissection and intraoperative stimulation furnishes substantial evidence of a segmental organization of functionally grouped nerve fibre bundles within the facial nerve A posterior branch represents the lower region of the face, an anteromedial bundle the upper region A minor difference between the two charts is found where the Chorda tympani is concerned Possibly the reason for this discrepancy is due to the fact that during surgery, the position of the Chorda can only be located by a lack of motoric responses following electric stimulation of the dissected fibre bundles

The results of our dissection experiments confirm a defined cross-sectional pattern already demonstrated in cats by May (1973) The feline and human organization patterns show a certain difference with respect to the olume of the nerve fibre bundles, however, ere is remarkable similarity regarding the interfascicular organization and the rotatory displacement of the main trunk The analysis of the fibre structure of the facial nerve within its meatal portion confirms the presence of a topic arrangement of fibre bundles described previously by Chouard et al (1972) In its tympanic and vertical segments however, wrapping of the superior branch by the fibres of the inferior branch could not be confirmed The discrepancy between our findings and the spatial pattern of fibre bundles described by Miehlke (1973) concerns only the topical orientation of the bundles within the nerve trunk

Summing up the results of our present study complete the picture of a functionally organized bundle grouping within the facial nerve trunk

Our observations are of some relevance with respect to facial nerve surgery in general and to the surgical treatment of hemifacial spasm in particular This clinical aspect will be discussed in a subsequent paper

ACKNOWLEDGEMENTS

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RÉSUMÉ

Les auteurs présentent une etude du nerf facial chez l homme qui ce base sur une série d'examens macroscopiques histologiques et électrodiagnostiques Ces données supportent l hypothèse de l existence d une orientation spatiale des fibres périphériques au niveau du tronc

ZUSAMMENFASSUNG

Die vorliegende Untersuchung befasst sich mit der Anatomie des Nervus facialis beim Menschen Sie beruht auf den Ergebnissen einer Anzahl von makroskopischen Faserverlaufsuntersuchungen am Leichenpraparat sowie auf denjenigen histologischer Schnittserien und elektrischer Reizung des Nerven bei operativen Eingriffen mit gleichzeitiger Registrierung des Summenaktionspotentials Die Ergebnisse samtlicher Untersuchungsmethoden konnen auf einen Nenner gebracht werden und liefern einen weiteren Beweis fur die Hypothese einer raumlichen Gliederung des Fazialisstammes in geordnete Faserbundel entsprechend der peripheren Aufteilung

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DISCUSSION

L B W Jongkees Can the described findings have any importance for nerve grafting?

S Podvinec (Reply) to Mr *Jongkees* In grafting the nerve with an implant not only the length but also the diameter must be considered because concentric as well as longitudinal shrinkage has to be considered as an important factor The adaptation of the proximal and distal stumps is more promising if they are refreshed by oblique splicing Finally the electric stimulation of fibre bundles as described in this paper gives further possibility to the surgeon to position the severed nerve properly following the described map of cross sectional organization of facial nerve fibre bundles

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REAKTIONSFORMEN DER MITTELOHRSCHLEIMHAUT

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Abstrakt Das histo-pathologische Schleimhautbild bei der Otitis media chronica adhaesiva ist geprägt durch das Nebeneinander akut entzündlich veränderter abheilender und narbiger Bezirke durch das Vorherrschen prolifera tiver entzündlicher Granulome im Speziellen der Cholesterol Granulome Infektabwehr und Restitution wer den von der Mittelohrschleimhaut allein bewältigt da die Unterstützung durch Drainage des Mittelohres mit Hilfe einer durchgängigen Tube und einer Trommelfellper foration, Grundbedingung für eine mögliche, vollkom mene Wiederherstellung bei Otitis media in diesem be sonderen Falle fehlt

Auf Grund eigener histomorphologischer und histo chemischer Untersuchungen an der Mittelohrschleim haut sowie cytologischer und biochemischer Unter suchungen am Paukenerguß gehört die Otitis media chronica adhaesiva zum Formenkreis der chronischen Otitis und unterscheidet sich nur graduell von der puru ten Form Sie kann sich aus jeder Otitis mit sterilem udat entwickeln und endet unbehandelt im Ad esivprozeß

Durch einen induzierten Gestaltswandel des klassischen Bildes der Otitis media auf dem Boden chemischer und antibiotischer Therapie *im Sinne Doerr's (1955), Link's (1961) und* Thiedemann's (1964) sind an die Stelle eitriger Komplikationen mehr und mehr die proliferativen, adhaesiven getreten Sie sind Ausdruck eines Gleichgewichts zwischen schädigender Noxe und Abwehrlage der Schleimhaut Vor bedingungen für das Entstehen der Otitis media chronica adhaesiva nach Zöllner (1942) dem Formenkreis der nicht eitrigen Mittelohrentzündung zugehörig, sind den Untersuchungen von Sirala, Ojala, Grahne, Singleton, Suehs u a m nach

1 Langer anhaltende mitigierte Entzündungen der Mittelohr- u Tubenschleimhaut, wobei ein keimfreier Erguß, wechselnder Konsistenz entsteht Man spricht von Otitis media acuta recidivans, Otosalpingitis schließlich Otitis media catarrhalis oder serosa

2 Hypovirulenz der Erreger, wodurch keine tiefgreifenden Zerstörungen an der Mittelohrauskleidung gesetzt werden Dies wird am ehesten durch die Wirkung der Antibiotica erklärt

3 Gestörte Tubenfunktion und fehlende Trommelfellperforation verhindern die Drainage des Mittelohrergusses und die Ventilation der Pauke

Die Otitis media chronica adhaesiva verläuft in Schüben Unbehandelt folgt auf die Initialphase mit der Ergußausbildung und dem Tubenblock bei intaktem Trommelfell das Stadium der Manifestation Die Mittelohr*schleimhaut sucht das angefallene Sekret zu* verarbeiten Da dies nur selten gelingt, entsteht in der Terminalphase der Adhaesivprozeß Er ist Ausdruck einer Defektheilung und führt zu beträchtlichen Hörstörungen Diesem Ablauf entsprechen typische Schleimhautbilder, wie wir an Hand eigener Untersuchungen zeigen konnten

PATHOLOGISCHE ANATOMIE

Nach Ojala und Singer wird die Mittelohrauskleidung erst durch den Reiz der Infektion zur Schleimhaut, nach Kessel (1892)

zum Sekretionsorgan Lim und Mitarbeiter (1970, 1971) sprechen darüber hinaus von einem mucociliaren, enzymatischen und immunologischen Schutzsystem Die Sekretbereitung geschieht in verschiedenartigen sezernierenden Zelltypen, der Sekrettransport erfolgt entlang der von Sade (1966) beschriebenen Flimmerstraßen Im Rahmen der Otitis media chronica adhaesiva reagiert die Schleimhaut, geprägt durch die Rezidivneigung folgendermaßen

1 *Subacute rezidivierende Entzündung mit seromucoser Sekretion (Otosalpingitis)*

Die Schleimhaut schwillt durch Verbreiterung der Lamina propria wegen Hyperämie und Oedem, sowie herdförmiger Rundzellinfiltration an Das Oberflächenepithel desquamiert stellenweise oder unterliegt der Metaplasie Die Zahl der Becherzellen nimmt vornehmlich zu Tiefgreifende destruktive Veränderungen fehlen jedoch Akut entzündliche Herde liegen neben in Abheilung begriffenen

2 *Chronische Entzündung mit Cholesterolgranulom (chronische Otitis media adhaesiva)*

(*a*) *Epithelveränderungen* An die Stelle des flachen Epithels tritt infolge Metaplasie und Hyperplasie über weite Strecken Zylinder Epithel zum Teil mit Flimmerbesatz und reichlich zwischengelegenen Becherzellen Da dieses Epithel Falten und Buchten überzieht und am Boden der Krypten besonders reichlich Becherzellen zu finden sind kann der Eindruck drüsenschlauchartiger Gebilde entstehen Eine andere auffällige Bildung sind die sogenannten Hohlraumsprossen ein Produkt der Epithelisierung von zwei Seiten her Zwischen hyperplastischen Epithelpartien ragen bindegewebige Proliferationsknospen in das der Schleimhaut aufliegende Exsudat hinein und leiten die Organisation bzw Verarbeitung dieses ein Am Rande dieser Sprossen kriecht das Epithel mit wodurch regelrechte Cysten entstehen können, ähnlich denen nach oberflächennaher Obliteration von Krypten Stellenweise findet man Epithel-Desquamation jedoch nur in bescheidenem Ausmaß Größere Epithel defekte konnten wir nicht beobachten

(*b*) *Bindegewebsveränderungen* Die entzündliche Infiltration der Lamina propria nimmt gegen die knöcherne Unterlage zu ab Sie ist bevorzugt subepithelial und perivascular gelegen Es herrschen Lymphocyten vor und Plasmazellen, Mastzellen fehlen nahezu vollständig, ebenso Granulocyten Die Rundzellen können granulomartig zusammentreten und dadurch Strukturen bilden, die Lymphfollikeln ähnlich sind Tiefgreifende Defekte, bzw echte Geschwürsbildungen fehlen Polypös proliferierende Herde treten zurück, während granulierende vorherrschen Das pathohistologische Substrat der Granulation ist vor allem das Cholesterol Granulom Es ist von einer bindegewebigen Kapsel umgeben enthält spaltförmige Hohlräume, die Cholesterinnadeln oder -platten enthalten und von Fremdkörperriesenzellen gesäumt sind Das dazwischenliegende Bindegewebsstroma ist wechselnd zell und faserreich Die Bindegewebsbrücken können auch atrophisch und schmal sein Sie bestehen dann fast nur aus verquollenen aufgebrochenen Fasern wodurch der Eindruck regressiver Veränderungen entsteht Fettfärbungen zeigen die starke Verfettungstendenz von Exsudat und Granulationsgewebe Auch das Epithel selbst kann reichlich Fettröpfchen enthalten Die Granulome atrophieren oder brechen auf und entleeren ihren Inhalt, entstanden durch homogene Nekrose, in das Mittelohr Bei diesen Vorgängen besonders aber bei Verarbeitungen des anfallenden Exsudates spielt neben der Organisation vor allem die fermentative Abbauleistung der Lamina propria eine große Rolle Verstärkte Aktivität unspezifischer Esterasen und der Leucinaminopeptidase ist vor allem in Granulomnähe nachweisbar Phosphatase Aktivität findet sich

bevorzugt im Bereiche verfetteter Cholesterol Granulome, im vernarbenden, unspezifischen Granulationsgewebe und in Herden dystrophischer Verkalkung

(c) *Mittelohrflussigkeit* Der Mittelohrerguß ist nach Sirala abakteriell Er besteht aus entzundlicher Exsudation, dem Sekret der metaplastischen Schleimhaut und den Abbauprodukten von organisiertem Exsudat und verfettetem Granulationsgewebe Neben Schleim finden sich bevorzugt Fettspaltprodukte und Cholesterin Beides wurde von Zechner und Mitarbeiter (1965) biochemisch nachgewiesen Im cytologischen Ausstrich fehlen Entzundungszellen Vorherrschende Zelltype ist der Makrophage, von denen viele nach Art der Bryan'schen Lipophagocyten aufgebaut sind Daneben finden sich auch Phagocyten, die mit Mucopolysacchariden und Ribonucleinsäuren beladen sind, was am deutlichsten bei Acridinorange fluorochromierung zu sehen ist

3 *Adhaesivprozeß (terminale Phase)*

Dieser ist Endzustand jeder chronischen Otitis, besonders ausgepragt aber nach adhaesiver chronischer Otitis media Das Epithel, welches das gekammerte und vernarbte Cavum tympani auskleidet, ist einschichtig zweireihig und nur ausnahmsweise herdformig hypertrophisch Die bindegewebige Unterlage ist immer verdickt, fibros induriert mit sparlichen, perivascularen Infiltraten durchsetzt Mitunter gelangen Herde dystrophischer Verkalkung zur Ansicht Zwischen Narbenzugen liegen resorptionscystenahnliche Raume und spaltformige Buchten, welche der Schleimhaut ein lochriges Aussehen verleihen konnen

ATIOLOGIE UND PATHOGENESE

Den Untersuchungen Siralas nach muß die stattgehabte Infektion eher schwach gewesen und im zweiten Stadium der Manifestation der chronischen adhaesiven Otitis media bereits abgeklungen sein Das Trommelfell ist nicht perforiert und die Tubenfunktion gestort Der Erguß im Mittelohr ist stets keimfrei, er bleibt liegen, ist zu Beginn seromucos, um später eingedickt u teilweise organisiert zu werden Ein Teil wird abgebaut und verflussigt und bildet mit dem Sekret des sezernierenden Schleimhautepithels das Suspensionsmedium fur das anfallende Cholesterin In dieser Suspension lassen sich immer zahlreiche Makrophagen nachweisen und wie wir zeigen konnten kaum Entzundungszellen Diese Ergußtype stellt einen wesentlichen Unterschied zwischen Otitis media chronica adhaesiva und purulenta dar

Das Bild einer Abortivform der Otitis mit Neigung zu Rezidiven ist sicher einerseits medikamentenabhangig worauf ja schon Mayer und auch Buchholz verwiesen haben Sie ist aber nach Lim (1974), Lim & Shimada (1971) Ishikawa et al (1972) Ausdruck einer besonderen immunologischen Situation Die Schleimhaut sezerniert Immunoglobuline nachgewiesen durch Howie et al (1973), Lim & Hussl (1969, 1970) Calseyde et al (1972) fanden, daß beim serosen Erguß = Typ 1, JgM u JgG, beim glue ear = Typ 2, JgA u JgM stark vermehrt sind und daß zwischen diesen Werten und den im Blut gefundenen kein Zusammenhang besteht Singer (1933) hat das subepitheliale Bindegewebe des Tympanon mit dem reticulohistiozytaren System verglichen und Lim u a bewiesen, daß die Schleimhaut sich wie ein eigenes Immunorgan verhalt

Die Haufung der Rezidive bringt eine Umformung der Schleimhaut mit sich Eigene Beobachtungen wurden von Gundersen & Gluck (1972), Tos & Bak-Pedersen (1972) Hentzer (1972) dahingehend bestatigt, daß es zu einer starken Zunahme der sezernierenden Schleimhautelemente kommt Weitere eingreifende Mucosaveranderungen bringt nach Politzer (1862), van Dishoeck (1941), Ingelstedt (1963) und Elner (1972) der Tubenblock Es fehlt die Druckregulation und aus dem normalerweise bestehen den milden Unter-

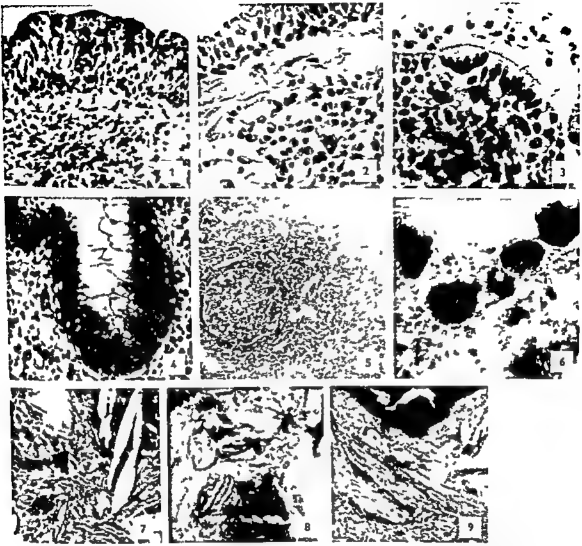

Abb 1 HE gefarbt akut entzundlich verandertes Schleimhautstuck (Biopsiematerial)

Abb 2 HE gefärbt chronisch entzundlich verandertes Schleimhautstuck mit stark verdickter Basalmembran (Biopsiematerial)

Abb 3 HE gefärbt herdförmige Zylinderzellmetaplasie mit Flimmerbesatz in chronisch entzundlicher Mittelohrschleimhaut (Biopsiematerial)

Abb 4 Sogenannte glandlike structure Anhaufung von sezernierenden Zellen am Kryptenboden dargestellt in MPSnachweisreaktion (Biopsiematerial)

Abb 5 HE gefarbt granulomartige an Keimzentren erinnernde Infiltratbildung in der Submucosa (Biopsiematerial)

Abb 6 Sudanfärbung Lipophagocyten aus dem Mittelohrerguß

Abb 7 HE gefarbt Cholesteringranulom zellreich

Abb 8 Esterasenachweis (dunkle Areale) und polarisiertes Licht Doppelbrechende Cholesterinkristalle und esterase positive Herde im Cholesteringranulom

Abb 9 HE gefärbt Cholesteringranulom z T verfettet z T narbig umgewandelt mit dystrophischen Verkalkungen

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Den Untersuchungen Siralas nach muß die stattgehabte Infektion eher schwach gewesen und im zweiten Stadium der Manifestation der chronischen adhaesiven Otitis media bereits abgeklungen sein Das Trommelfell ist nicht perforiert und die Tubenfunktion gestort Der Erguß im Mittelohr ist stets keimfrei er bleibt liegen ist zu Beginn seromucos um spater eingedickt u teilweise organisiert zu werden Ein Teil wird abgebaut und verflussigt und bildet mit dem Sekret des sezernierenden Schleimhautepithels das Suspensionsmedium fur das anfallende Cholesterin In dieser Suspension lassen sich immer zahlreiche Makrophagen nachweisen und wie wir zeigen konnten kaum Entzundungszellen Diese Ergußtype stellt einen wesentlichen Unterschied zwischen Otitis media chronica adhaesiva und purulenta dar

Das Bild einer Abortivform der Otitis mit Neigung zu Rezidiven ist sicher einerseits medikamentenabhangig worauf ja schon Mayer und auch Buchholz verwiesen haben Sie ist aber nach Lim (1974) Lim & Shimada (1971) Ishikawa et al (1972) Ausdruck einer besonderen immunologischen Situation Die Schleimhaut sezerniert Immunoglobuline nachgewiesen durch Howie et al (1973) Lim & Hussl (1969 1970) Calseyde et al (1972) fanden daß beim serosen Erguß = Typ 1 JgM u JgG beim glue ear = Typ 2 JgA u JgM stark vermehrt sind und daß zwischen diesen Werten und den im Blut gefundenen kein Zusammenhang besteht Singer (1933) hat das subepitheliale Bindegewebe des Tympanon mit dem reticulohistiozytaren System verglichen und Lim u a bewiesen daß die Schleimhaut sich wie ein eigenes Immunorgan verhalt

Die Haufung der Rezidive bringt eine Umformung der Schleimhaut mit sich Eigene Beobachtungen wurden von Gundersen & Gluck (1972) Tos & Bak Pedersen (1972) Hentzer (1972) dahingehend bestatigt daß es zu einer starken Zunahme der sezernierenden Schleimhautelemente kommt Weitere eingreifende Mucosaveranderungen bringt nach Politzer (1862) van Dishoeck (1941) Ingelstedt (1963) und Elner (1972) der Tubenblock Es fehlt die Druckregulation und aus dem normalerweise bestehen den milden Unter

tant than purulent complications The middle ear drainage normally provided by good function of the eustachian tube and a drum perforation have now become a focus of interest A blocked tube and an intact eardrum cause not only serous otitis media but when untreated otitis media chronica adhesiva as well We have studied these problems histologically histochemically and cytologically

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DISCUSSION

R Albrecht Meine Frage geht an alle drei Vortragenden Hr Arnold wies in der Schleimhaut bei Otosklerose auf Kalziumapatitformationen im Gewebe hin Demnach interessiert es mich ob Hr Zechner und Frau Wullstein welche auch auf kalzifizierte Substanzen hinwies diese als Ca Apatit nachweisen konnten Wir wissen dass bei Sialolithiasis ein Teil der kleine Apatitsteine sind sodass Ckran entzundete Schleimhäute hier vielleicht Gemeinsamkeiten zeigen, einschliesslich die Otosklerose?

T Palva You suggested that the degenerating cholesterol granulomata transform into fatty tissue What evidence is there to this? Could it not be the other way round, similar to macrophage containing cyst transforming into cholesterol granulomata? Then about the enzymes I think that their main activity can be seen in the epithelium more than in the stroma The greatest activity is seen with the phosphatases and dehydrogenases Are there any particular reasons for ascribing non specific esterases a specific function with the cholesterol granulomata?

C R Pfaltz Which is the proof of the hypothesis that the alterations of the middle ear mucosa are mostly due to a previous antisiolic treatment, modifying the immunological reactivity of the tissue? Since similar histologic findings have been published already before the chemo therapeutic and antibiotic area, other factors such as middle ear ventilation and drainage must also be taken into consideration as the cause of these pathologic reactions of middle ear mucosa

F Escher Does the transformation from a cholesterolgranuloma in a real tympanosclerosis with hyalinization and calcification happen often? Do you agree that an intervention for aeration should be done as early as possible?

G Zechner (Reply) to Frau *Albrecht* Die Kalkablagerungen sind meines Erachtens dystrophische Kalcification

To Mr *Palva* Fatty degeneration takes place, the fatty tissue islands subside necrotic cholesterolgranulomas, if they get contact with the middle ear cleft Enzyme activity is shown in the epithelium, but also in the granulation tissue

To Mr *Pfaltz* The antibiotic therapy is one fact only because of influencing the local immunological conditions

To Mr *Escher* Cholesterolgranuloma is only one typical tissue formation Necrosis or hyalinization is the curriculum What we can do practically is to cure this form of otitis early restoration of middle ear clearance

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VERANDERUNGEN IM BEREICH DER MITTELOHRSCHLEIMHAUT BEI DER OTOSKLEROSE

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Abstrakt Untersuchungen von Schleimhautproben von 98 Otosklerosekranken erbrachten regelmaßig mesenchymale vaskulare und epitheliale Veranderungen gegenuber dem normalen Aufbau der Mittelohrschleimhaut im ovalen Fensterbereich und Promontonalbereich Diese Veranderungen bestehen in einer Einlagerung von Mucopolysacchariden in das kubisch umgewandelte Epithel welches zudem im Cytoplasma Tonofilamente Glykogen und an seiner Oberflache pathologisch angeordnete Kinozilienbundel zeigt

Bei der sogenannten roten Otosklerose kommt es im Bereich der Submucosa zu einer Destruktion der kollagenen Faserstrukturen im Sinne einer Fibrolyse und spateren Hyalinose sowie zu einem exozytoschen Untergang der Fibrozyten Die Kapillaren sind stark mit gestauten Erythrozyten angefullt und zeigen perivaskular Fibrinausfallungen Daneben findet man zahlreiche Kapillaren im Zustand der Obliteration Insgesamt ist die Submukosa stark verbreitert auf Serienschnitten von Felsenbeinen hat man den Eindruck als ob diese Verbreiterung auf Kosten einer Knochenresorption zustande kommt Vielerorts findet man sowohl im freien Extrazellularraum als auch entlang der Kollagenfasern eine Ausfallung von Kalziumapatitkristallen oftmals zu dichten Kalkdrusen

Bei der sogenannten weißen Otosklerose ist die Submukosa zu einer Narbenplatte umgebaut (Hyalinose) mit kreuz und quer verlaufenden unreifen Kollagenfasern Gefaße fehlen weitgehend Zarte Knochenbalkchen konnen subepithelial beobachtet werden

Unsere Untersuchungen lassen den Schluß zu daß die auslosende Ursache des Schleimhautumbaues in einer Gefäßstauung und Obliteration von Kapillaren zu suchen ist Die daraus folgende Sauerstoffverarmung des Gewebes erklart die beschriebenen Ab und Umbauvorgange des mesenchymalen Gewebes Es wird vermutet daß auch der Knochenumbau bei der Otosklerose unmittelbar abhangig von den pathologischen Veranderungen an den Gefäßen ist

Bei der Betrachtung histopathologischer Schnittserien otospongioser und otosklerotischer Prozesse findet man mit erstaunlicher Regelmaßigkeit eine direkte Abhangigkeit des Knochenumbauprozesses vom vorhandenen Gefäßbild Wahrend im otospongiosen also im aktiven oder Erweichungsstadium der Otosklerose die Gefaße extrem weit gestellt und thromboseahnlich mit Erythrozyten ausgefullt sind entdeckt man im otosklerotischen Stadium welches als End oder Vernarbungsstadium aufzufassen ist, kaum mehr blutfuhrende Gefaße Aus dieser Beobachtung haben bereits manche Untersucher den Schluß gezogen daß die Vaskularisation bei der Otosklerose einen unmittelbaren Einfluß auf den Umbau des Knochens habe (Nager & Meyer 1932, Wittmaack 1919 1931 1933)

An der Frankfurter und Tubinger Universitatsklinik wurden bei 94 Otosklerosepatienten wahrend der Operation Probeexcisionen der Mittelohrschleimhaut entnommen und diese licht und elektronenmikroskopisch aufgearbeitet (Methode siehe Arnold & Plester 1975) Dabei fanden wir absolut regelmaßig folgende charakteristische Veranderungen gegenuber dem normalen Schleimhautaufbau

1 Eine Hamostase in vielen Gefäßbereichen und zahllose obliterierte Kapillaren mit typischen Basalmembranveranderungen im Bereich der Schleimhaut Knochengrenze (Abb 1 2)

2 Um die Kapillaren herum kommt es zu einem Ab und Umbau der typischen kollagenen Struktur im Sinne einer Fibrolyse und


4
1
S
3
2
Abb.1


C
Abb.2

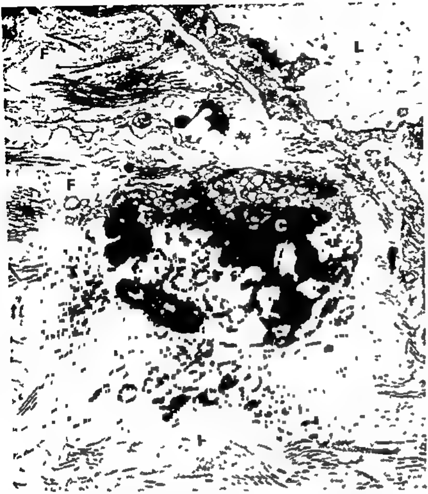

Abb. 3. In der Umgebung einer stark gestauten Kapillare mit dichten eiweißähnlichen Niederschlägen im Lumen (*L*) erkennt man neben einer Destruktion des Kollagens Fibrinablagerungen (*F*). *C* exocytotischer Untergang eines Fibrozyten mit Einlagerung von Kalziumphosphat. ×9800

späteren Hyalinose, nicht selten findet man im perivaskulären Einzugsgebiet der gestauten Gefäße eine Fibrinausfällung mit elektronenmikroskopisch sichtbarer typischer Querstreifung (Abb. 3). Der regelmäßig zu erkennende exozytotische Untergang vieler Matrixzellen des Kollagens (Fibrozyten) bedingt eine Ausstoßung von Zellorganellen, vorzugsweise von Lysosomen. Offensichtlich ist auch eine überschießende Kollagenproduktion möglich, jedoch in Form von unreifen prokollagenen Fasern. Sowohl im freien Extrazellularraum als auch an den unreifen Kollagenfasern lagern sich nadelförmige Kalziumapatitkristalle ab (Abb. 4).

3. Insgesamt führt der Umbau der Mittelohrschleimhaut zu einer starken Zunahme der Breite des subepithelialen Raumes und

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Abb. 1. 450fache Vergrößerung eines Sagittalschnittes durch den vorderen Stapesschenkel. EDTA-entkalkt. *1* gestautes, stark erweitertes Gefäß, *2* obliteriertes Gefäß, *3* gegenüberliegende normale Schleimhautkontur, *4* metaplastische Epithelinseln mit Kinozilienbesatz

Abb. 2. 17640fache Vergrößerung einer obliterierten Kapillare aus der Submukosa über einem otosklerotischen Herd der Stapesfußplatte. Neben der vielschichtigen, schlierig verbreiterten Basalmembran kommt es in der Umgebung des Gefäßes zu einem lytischen Untergang der Fibrozyten und Destruktion des Kollagens. *C* Kalziumphosphateinlagerungen in dem untergehenden Cytoplasma eines Fibrozytenfortsatzes. *E* untergehende Endothelzelle mit Verlust jeglicher Cytoplasmastruktur

b 4 Versch edene Stad en des Kollagenumbaues PK okollagene Fasern K re fe Kollagenfasern E ne regu lare Ausrichtung der kollagenen Fasern ist n cht mehr zu erkennen Neben der F brolyse m t Aufschlusselung der Kollagentextur erkennt man bere ts e ne beg nnende Hyal n s erung m t ungeordnetem Verlauf der Fasern C ungeordnete Ablagerungen von Kalz umapa kristal len entlang und zw schen den kollagenen Fase ma sen ×16200

zwar auf Kosten einer periostalen submu kosen Knochenresorption Dies erkennt man sehr deutlich an der Zunahme der sogenann ten periosteozytaren Osteolyse im Bereich der Schleimhaut Knochengrenze sowie in einer starken Entkalkung und somit De maskierung der kollagenen Fasern des Kno chens in dieser Grenzzone Im Zentrum sol cher Entkalkungszonen liegt stets ein obli teriertes oder gestautes Gefäß (Abb 5a b) Elektronenmikroskopisch zeigen diese de maskierten Kollagenfasern deutliche Veran derungen im Sinne von endstandigen trom melschlegelahnlichen Auftreibungen wie sie ahnlich von Chevance et al (1970) im Be reich der sogenannten knochernen Mikro foci als Pasteur Pipetten Form beschrieben wurden

4 Schließl ch ist das normalerweise flache einschichtige zilienlose Epithel der Mittelohr schleimhaut bei der Otosklerose zu einem kubischen Epithel mit Einlagerungen von PAS positiven Einschlussen umgewandelt und ze gt zudem eine gebundelte atypische Anordnung der Kinozil en haufig findet man im Cytoplasma der Epithelzellen (bevorzugt

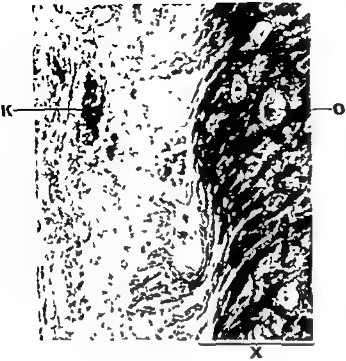

Abb 5a 960fache Vergroßerung der Grenzzone zwischen Schleimhaut und Knochen In diesem Bereich erkennt man eine deutliche Demaskierung der kolla genen Fasern des Knochens welche un regelmaßig und mit Auftreibungen in die Submukosa einstrahlen Im großtenteils be reits entkalkten ehemaligen Knochen bereich (×) erkennt man zahlreiche Osteozytenhofe im Stadium der periosteocy taren Osteolyse (O) K obliterierte Kapil lare

bei der sogenannten weißen Otosklerose) Tonofilamente, wie sie gewohnlich im Epithel der Mittelohrschleimhaut nicht beobachtet werden (Abb 6*a*, *b*)

Es erhebt sich die Frage, inwieweit die be schriebenen Schleimhautveranderungen in direkter Beziehung zum otospongiosen oder otosklerotischen Herd stehen Eine Durch sicht der Wittmaack schen Felsenbeinsammlung in Hamburg zeigte uns, daß die Schleimhaut uber einem aktiven otospongiosen Umbauprozeß regelmaßig um das Zehn bis Zwanzigfache verdickt ist das Epithel mehr schichtig kubisch umgewandelt ist und fast alle Gefaße Zeichen einer Stauung oder Obli teration aufweisen Der submukose Raum ist gegen die bindegewebig umgebauten Resorp tionslakunen des darunterliegenden otospongiosen Knochenherdes nicht mehr scharf abzugrenzen Eine direkte Verbindung zwi schen den Gefaßen des Knochenumbauherdes und denen der Mittelohrschleimhaut wird deutlich (Abb 7*a b*)

Dem Mittelohrchirurgen ist diesbezuglich die auffallend starke varikose Gefaßzeichnung bei der sogenannten roten Otosklerose bekannt, ein Befund, der auch Schwartze-sches Zeichen genannt wird Diese varikose Gefaßzeichnung beruht auf der histologisch erkennbaren Hamostase der Schleimhautgefaße im Einzugsgebiet eines otospongiosen Umbauprozesses

Die Schleimhaut uber Knochenherden, deren Umbau bereits abgeschlossen ist—wir mochten sie als echte Otoskleroseherde bezeichnen—imponiert weiterhin als stark ver dickt ist jedoch in der Regel weitgehend gefaßlos und zeigt einen vollig ungeordneten, geflechtartigen Verlauf der kollagenen Fasern Nach wie vor sind die direkten Verbindungen zwischen fibros umgewandelten Restlakunen des Otoskleroseherdes und der narbenahn lich verdickten Submukosa deutlich zu sehen (Abb 8) Gelegentlich findet man un mittelbar unterhalb des Epithels Mikroossi fikationsherde mit lamellarem Knochen- sie bereits von C

Abb 5b 6840fache Vergroßerung der gleichen Region wie in Abb 5a Die unscharfe lakunenförmige Grenzzone zwischen Submukosabezirk (*S*) und knöcherner Randzone wird deutlich Die kollagenen Fasern (*K*) brechen plotzlich ab die Osteozytenhofe sind verbreitert und haufig findet man einen exocytotischen Untergang der Osteocyten (*LO*)

zuvor schon vom Wittmaack (1919) beschrieben wurden Daneben macht die elektronenmikroskopische Auswertung zahlreiche Neuformationen von Kalziumapatitablagerungen an Kollagenbündeln oder plumpe Ausfällungen von amorphen Kalkdrusen ohne typische Bindung an kollagene Fasern deutlich Der vormals weite, flüssigkeitsgefüllte Extrazellularraum ist verschwunden und durch kollagene Fasermassen ersetzt (Abb 9) Der verdickten Submukosa fehlt in diesem Stadium des Umbaues jeglicher freie Extrazellularraum da-

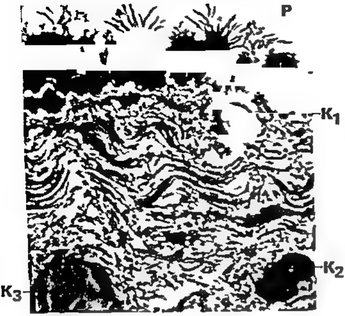

Abb 6a 1450fache Vergroßerung e nes Ausschnittes der M ttelohrschle mhaut uber einem otosklerot schen Herd des Promontoriums *P* Paukenlumen *K* einge engte Kap llare *K₂* obliterierte Kapillare *K₃* stark gestaute m t e we ßahnl chem Material ausgefüllte Kap llare Dazwischen die unregelmaß ge unterbrochene Kontur der Kollagenbundel Das Epithel st ku b sch und ze gt e ne ungewohnl che ge bundelte Anordnung der K nozil en

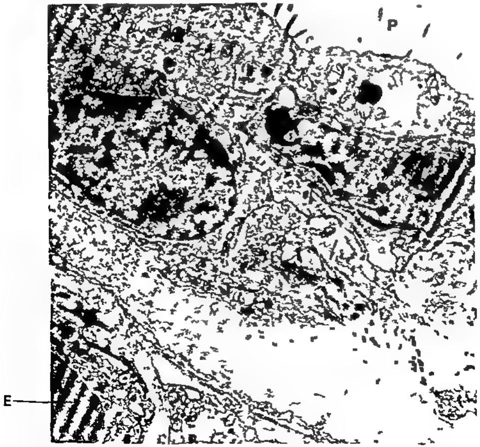

Abb 6b 9800fache Vergrößerung aus dem Schle mhaut bere ch uber e nem otosklerotischen Herd am Rande der ovalen Fensternsche *P* Paukenlumen *E* Erythrozyt in einer e ngeengten Kap llare mit stark verbre teter Basalmembran (→) Tonof lamentbündel in e ner Basalzelle

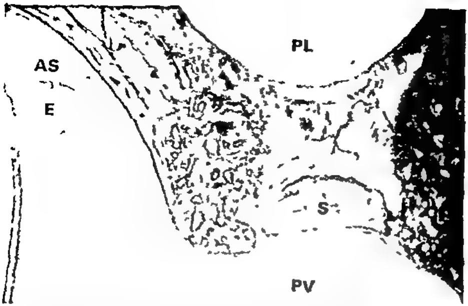

Abb 7a 30fache Vergroßerung eines Horizontalschnittes *durch die ovale Fensternische (Wittmaack sche Sammlung* Hamburg) Uber der bereits otosklerotisch umgebauten Fußplatte (*S*) findet sich eine enorm stark verbreiterte Schleimhautschicht mit gestauten Kapillarknauel *PL* Paukenlumen Am vorderen und hinteren Anteil der ovalen Fensternische erkennt man aktive otospongiose *Herde* (→) aus dem hinteren Herd zieht ein gestautes Gefaß in die Mittelohrschleimhaut ein *Pl* Perilymph raum des Vestibulums *E* Endolymphe *AS* atrophierte Stria vascularis der Basalwindung

neben kommt es zu einem volligen Schwund der Blutkapillaren und Lymphgefäße Wir betrachten dieses Stadium als Narbenstadium, ter dem Operationsmikroskop ist dieser hleimhauttyp glasig verdickt, weißlich glännd und gefäßlos

DISKUSSION

Die aufgewiesenen Veranderungen an den Gefäßen und die charakteristischen mesen chymalen Umbauvorgange konnten wir an allen unseren Probeexcisionen von Otosklerosekranken *nachweisen Daraus muß* zwangslaufig geschlossen werden daß die pathologische Reaktionsweise der Gefäße mit der Erkrankung im unmittelbaren Zu sammenhang *gesehen werden muß Die Stase* in vielen Gefäßabschnitten und die Obliteration der Kapillaren sehen wir als pathogenetischen also auslosenden und somit primaren Faktor bei der *Otosklerose* an *Durch* diese Stase und Gefäßobliteration kommt es zu einer Ischamie im perivaskularen Ge webe Es ist wahrscheinlich, daß es durch die entstehende Azidose (pH-Verschiebung) und die daraus resultierende Fermententglei sung zu einer Loslosung der Kalziumphos *phatkomplexe von den kollagenen* Fasern im Bereich der Schleimhaut Knochen grenze kommt Im ossaren Bereich wird zudem die gleiche Ursache zu einer Ernahrungsstorung *der Osteozyten fuhren, wodurch sich* die vermehrte periosteozytare Osteolyse erklart Alle beschriebenen Veranderungen im Schleimhautbereich, wie Kollagenabbau und -umbau *sowie exocytotischer Untergang der* Fibrozyten lassen sich als normale Folge der Gewebsubersauerung bei einer Ischamie erklaren

Bezuglich der Gefäßveranderungen und der perivaskularen Umbauvorgange ist auch auf die Untersuchungen von Chevance et al (1970) *zu* verweisen welche elektronen *mikroskopisch in unmittelbarer Nahe der* Gefäße eine Fragmentation der kollagenen

Abb. 7b. Ausschnittvergrößerung aus Abb. 7a. 140fach vergrößert. *PL*: Paukenlumen. (→) Direkte Verbindung gestauter Gefäße des otospongiösen Herdes mit den gestauten Kapillaren der Mittelohrschleimhaut

Fasern im otosklerotischen Knochen fanden. Topographisch handelt es sich nach Chevance bei diesen Regionen um die lichtmikroskopischen Bezirke der sogenannten blauen Mantel. Nach Ansicht der genannten Untersucher kommt es im ersten Stadium der Otosklerose, wahrscheinlich aufgrund einer Enzymentgleisung zu einer Lyse des Knochens. Auch andere Autoren vermuten, daß erst das Zusammenspiel zahlreicher pathologischer Enzymreaktionen (Alberti & Tarkannen 1963, Holdsworth et al. 1973, Albernaz & Covell 1961) zu einer grundlegenden Veränderung der Textur des kollagenen Bindegewebes und der Matrixzellen des Knochens führt.

Von diesen Gesichtspunkten geleitet glauben wir, daß die Ursache der Fermententgleisungen und der davon wiederum abhängigen pathologischen Bindegewebsreaktionen bei der Otosklerose als unmittelbare Folge der Gefäßobliterationen zu verstehen ist.

Die von Wullstein et al. (1960) gefundene Zunahme der alkalischen Phosphatase in der Perilymphe erklärt sich durch die unmittelbare Beziehung der otospongiosen Resorptionslakunen zu den Perilymphräumen, wie wir sie an vielen Felsenbeinpräparaten mit Otosklerose aus der Wittmaack'schen Sammlung (Hamburger Universitätsklinik) studieren konnten. Der Diffusion lysosomaler Enzyme von den Knochenumbauherden zu den Perilymphräumen ist hier keine Schranke mehr gesetzt. Ein Abfluß zur Mittelohrschleimhaut ist ebenfalls wegen der Verödung der dort normalerweise vorhandenen breiten Lymphabflußwege durch den Narbenprozeß nicht mehr möglich (vergl. Arnold & Vosteen, 1975). Das gleiche Argument trifft für die Beobachtungen von Chevance (1975) zu, welcher fand, daß in der Perilymphe von Otosklerosekranken die Trypsinaktivität erheblich erhöht ist. Auch diese Kollagenase diffundiert aus dem otospongiosen Herd in die Perilymphe. Es wäre verständlich, eine Erklärung der Innenohrsymptomatik (Tinnitus, Schallempfindungsverlust in den hohen Frequenzen, Schwindel) bei der Otosklerose auch in der andauernden Zufuhr von toxischen Substanzen aus der Mittelohrschleimhaut und aus den Umbauherden in die Perilymphe zu suchen. Daß es daneben auch, wie Ruedi (1965), Gussen (1975) und Linthicum et al. (1975) zeigen konnten, zu einer direkten vaskulären Beeinflussung der Innenohrdurchblutung, abhängig von der Lokalisation des Otoskleroseherdes kommen kann (Untergang von Kapillaren und Atrophie des Ligamentum spirale) fügt sich zwanglos ein in unser Konzept der Durchblutungsstörung und Obliteration von Gefäßen als pathogene

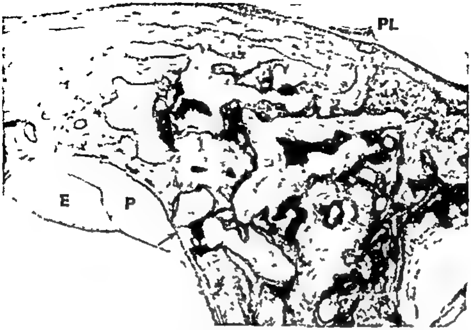

Abb 8 30fache Vergroßerung eines otosklerotischen Herdes welcher sich vom Bereich der Submukosa bis zum perilymphatischen Raum durch die gesamte Labyrinthkapsel erstreckt Es besteht direkter Kontakt zum perilymphatischen Lumen *(P→) PL* Paukenlumen *E* Endolymphe

tisch ausschlaggebender Faktor bei der Otosklerose

Im Grunde genommen haben wir durch unsere Untersuchungen nur Wittmaacks Theorie bestatigt daß namlich der otosklerotische Erkrankungsprozeß auf einem Stauungsvorgang (Wittmaack glaubte an einen rucklaufigen Blutstrom nach Art der Varizenbil

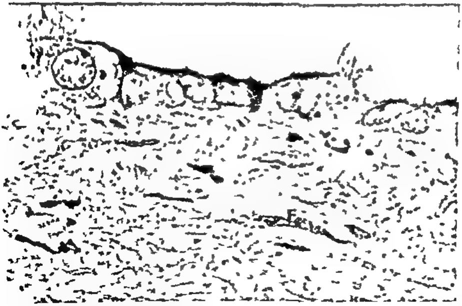

Abb 9 960fache Vergroßerung eines Schleimhautausschnittes uber einem otosklerotischen Herd des Promontoriums Neben der Epithelmetaplasie mit osmiophilem *und PAS positivem* Material im Cytoplasma zeigt der submukose Bereich *(F)* ein ungeordnetes dichtes Netzwerk von kollagenen Fasern und nur wenige Fibrozyten *(F)*

dung) innerhalb der Gefäßbahnen der befallenen Bezirke beruhen musse Die Frage, wie es zu dieser venosen Stase oder zum Auftreten eines rucklaufigen venosen Blutstromes innerhalb der einzelnen Gefäßversorgungsbezirke der Labyrinthkapsel kommt und was die Ursache der Gefäßobliteration ist, bedarf noch der Beantwortung Allerdings gibt es bereits einige plausible Erklarungsversuche, welche die Atiologie der Gefäßveranderungen beantworten konnten

1 Labile Gefäßbezirke im Bereich der Fissula ante Fenestram und der Labyrinthkapsel vorwiegend in der Nischengegend aber auch in anderen cochlearen Bezirken (Bast & Anson, 1949, Portmann, 1942, Wolff, 1949, Fowler & Fowler, 1950)
2 Eine Irritation des labilen Gefäßkomplexes, insbesondere am Ubergang vom Innen zum Mittelohr innerhalb eines topographischen Bezirkes, der nach den Untersuchungen von Otto Mayer (1930) Šercer (1958), Šercer & Krmpotic (1958, 1960) wahrend der ontogenetischen Entwicklung besonders starken Druck Zug Dreh kraften ausgesetzt ist

RÉSUMÉ

L examen de la muqueuse de l oreille moyenne dans des cas d otospongiose a permis de constater des modifications importantes des capillaires Dans leur voisinage immediat on peut observer exocytose fibrolyse du collagène et souvent transformation du collagene en substance hyaline des dépots de cristaux calcaires un précipité libre de calcium et des accumulations des glycocène dans les fibrocytes

SUMMARY

In 98 samples of middle-ear mucosa taken from patients during otosclerotic stapes surgery considerable pathological changes in the organization of the capillaries were found (endothelial hydrops proliferation of the intima and adventitia total obliteration of small vessels) They caused pathological reactions of the mesenchymal elements of the submucosa fibrocytes undergo lysis collagen fibres show fibrolysis and hyalinosis Free and cellular bound calcium deposits are located in these lytic areas Even the epithelial stratum reveals uncommon inclusions such as glycogen and acid mucopolysaccharides The basement membranes of the capillaries as well as of the epithelium show enlargements and solution of their ultrastructure The changes of the connective tissue and of the epithelium are secondary to the capillary obstructions

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DISCUSSION

H Spoendlin According to your opinion otosclerosis is initiated by ischaemia due to congestion and obliteration of blood vessels There is however according to our histopathological studies in 90 otosclerotic temporal bones a great increase in the number and not only congestion of blood vessels in active otosclerotic foci Your findings might be the consequence of the maturation of the otosclerotic focus rather then the initiating process Ischaemic bone formation is known to exist in the inner ear but it is of a different type than otosclerotic bone

G Zechner Ich darf an Hand eigener Schnitte Ihre usführungen unterstützen Auch im Labyrinthknochen mmt es zu einer praparativen Zone mit Veranderung r kollagenen Fasern und der Grundsubstanz Welche olle spielen Ihrer Ansicht nach d e sauren Mucopoli acchande?

H Kristensen In our attempt to find the factor responsible for breakdown of bone in otosclerosis we found along with Chevance enzymatic factors A similar finding we had in chronic otitis media In both cases we gather hyperemia is one provocative factor How can this be explained compared with changes in mucous membrane?

M Arslan Diese Befunden sind sehr wichtig als Beweis der Hypothese dass die Otosklerose eine lokale Kollagenose (mit anderen Worten Mesenchymopathie) ist Eine ahnliche Krankheit ist die Osteogenesis im perfecta

W Arnold (Antwort) zu Hr *Spoendlin* Die scheinbare [illegible]

Zunahme der Gefassquerschnitte kommt ist deshalb schwer zu beobachten da Untersuchungen uber d e normale Vascularisation des Labyrinthknochens fehlen Man weiss allerdings dass derartige Stauungsvorgange normalerweise in der Labyrinthkapsel nicht vorhanden sind

Zu Hr *Zechner* Die Bilder machen deutlich dass der Schleimhautumbauprozess in unmittelbaren Beziehung zum otospongiosen Herd steht Appositionelles Knochenwachstum im Bereich der Submucosa findet man gelegentlich im Spat oder Vernarbungsstadium der Otosklerose meist jedoch bleibt die Schleimhaut Knochengrenze unregelmassig mit tiefen bindegewebig umgebauten Resorptionslakunen bestehen Aus der allgemeingen Rotfarbung einer submucosen Zone (Eosinophilie) kann man nicht schliessen dass es sich dabei um einen Knochenanbau oder Abbauprozess handelt (Wittmaack)

Zu Hr *Kristensen* Wahrend man bei der chronischen Otitis media Zellen der immunologischen Abwehr H stiocyten Plasmazellen Mastzellen) findet fehlen diese in der Schleimhaut Otosklerosekranker Die Stauung in den Gefassen bei der chronischen Otitis ist durch die aktive Hyperamie bei einer chronischen Entzundung bedingt Zweifelsohne ist auch im Narbenstadium einer chronischen Otitis media eine hyaline Umwandlung möglich insgesamt handelt es sich dabei um den Reaktionsablauf einer Periostitis

Zu Hr *Arslan* Wir haben versucht Wittmaacks klassische aber nie richtig verstandene Vorstellung die Otosklerose sei eine Knochenerkrankung als Folge einer pathologischen Gefassstauung (Stase) anhand der dokumentierten Veranderungen an der Mittelohrschleimhaut zu beweisen Es kommt als Folge des Ischamie zu typischen mesenchymalen Veranderungen wie man sie auch bei Kollagenosen (Rheumatismus Schwielenbildung etc) sieht Ich glaube aber nicht dass ein primarer Enzymdefect (Chevance) vorliegt sondern vielmehr dass die Veranderungen des Kollagens sekundar bedingt sind durch eine Ernahrungsstörung (O_2-Mangel) der Fibrocyten Dies fuhrt naturlich zu enzymatischen Ab und Umbauvorgängen wie sie bei der Otosklerose bekannt sind

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HISTOPATHOLOGICAL ALTERATIONS OF THE MUCOSAL FOLDS IN CHRONIC OTITIS MEDIA

Sabina F Wullstein

From the ENT Department of the University of Wurzburg Wurzburg BRD

Abstract The autogenesis and evolution of the mucosal folds were studied They play an important part in the immunological defense system of the middle ear The anatomy and topography of these mucosal duplications together with their pathophysiological effects explain the clinical development in the various forms of middle ear infection and disturbances of the aeration of the middle ear in case of an open and well functioning Eustachian tube

My topic today is the histopathological alterations in the mucosal folds anatomically and topographically constituting a specific component of the middle ear which I believe has received too little attention over the years

Perhaps this is due to the difficulty of demonstrating the mucosal folds as long as they are unaffected by disease, since we do not operate on the healthy ear In dissection of an ear post mortem, the folds dry out rapidly on exposure to the air In a pathologically changed situation they are partly destroyed by the focus of disease itself and partly by the surgeon during his surgical manipulation

One has rarely been concerned with these folds in spite of their having been described in the greatest of detail a long time ago We are absolutely sure of their not being an artefact or a coincidental finding and not even the result of a preceding infection They are an important structure of the middle ear, as shown by Hammar s studies, dating back to the turn of the century and those of B Proctor, which appeared during the era of tympanoplasty Their origin begins with the onset of late fetal and early postnatal development Their shapes, location and prevalence are the result of either normal or disturbed bone growth and airspace formation during that period

The mucosal folds represent the direct continuation of the respiratory type mucosa of the middle ear They extend around the ossicles and reach the walls of the epitympanum similar to a mesentery, usually in those places where structures of the middle ear obstructed their course of growth during the fold s developmental stage the chorda tympani the tendons of the tensor tympani and stapedius muscles, the ossicles and their ligaments vessels and other prominences and eminences of the middle ear relief Here delicate duplications of the mucosal lining of the middle ear evolve from the four sacci as the ear is aerated In this way compartments originate around the ossicles which I imagine as ' air cushions' , whose function has not yet been fully defined However these folds can easily be construed as being partly responsible for maintaining an equalized air pressure in the epi and retrotympanic spaces

On both sides the mucosal folds are covered with an active respiratory type of epithelium and depending on the location where the folds lie and how closely they associate with the ventilation and drainage pathways, they contain flat, cuboidal, non ciliated and ciliated columnar epithelium containing goblet

cells This causes the actively effective surface of the mucosal lining of the middle ear to enlarge considerably.

Also, because of their very loose and well vascularized stroma the folds participate in the immune defence system of the middle ear We are able to observe in them all the well known morphological alterations described in the mucosal lining of the middle ear, according to the degree of inflammation, the virulence of the infecting agent, the condition of the immuno-biological defence action of the body *and to no lesser extent to the size of the air-filled middle ear volume* and to the form of the middle ear relief which all together influence these changes

The first and foremost important reaction of the system of folds to pathological disturbances in the middle ear is the increase in the amount of fluid in the loose stroma congestion, oedema and cellular infiltration The thickening of the folds themselves and the simultaneous decrease in the size of the air-containing space, soon to fill with secretion, lead primarily to a narrowing and later on to an obstruction of the air-passages to the epitympanum and the antrum As a consequence drainage from this area is effectively stopped

The interruption of the air flow, so necessary of the respiratory type mucosa in the middle ear, and at the same time the interruption of drainage, resulting in an increased secretory activity of the epithelium, causes an accumulation of secretion inside the "compartments" and an insufficient "clearance" of the middle ear With a consequent negative pressure present in the retrotympanal spaces, new irritation to the tissue occurs and further morphological alterations and consequential effects are induced

The histopathological changes I am showing you today have been obtained exclusively from the mucosal folds which I systematically removed during osteoplastic epitympanotomy, from 1971 to 1975, due to chronic middle ear infections The age of the patients ranged from 4 to 65 Specimens were removed from different topographical sites, mostly from the lateral incudal fold or superior malleolar and incudal folds and from lateral malleolar folds Serial sections were embedded in paraffin and stained with haematoxylin eosin, Alcian blue, periodic acid Schiff, Goldner, Phloxyin–Alcian blue–Orange, von Kossa In some cases many histological serial sections were frozen and stained with haematoxylin-eosin, PAS, scarlet red

In chronic suppurative inflammation of the middle ear, mucosal changes are constant and *widespread and affect both epithelium* and underlying stroma of the mucosal lining of the mesohypotympanum as well the epitympanum with the system of the folds and the whole air cell system of the mastoid Therefore, the mucosal lining of the tympanic cavity should not be investigated separately from the mucosal folds, because they constitute an "all-in one" anatomical structure displaying morphological alteration of varying extent and at different levels of activity, according to the stage of the illness

The epithelium of the folds shows an increase of ciliated and secretory cells hyperplasia, squamous metaplasia, glandular metaplasia as a result of invagination into the underlying stroma In this way, gland like formations occur, filled with a substance secreted by the epithelium they are lined with, marking all stages of secretion up to total obstruction and formation of large cysts Keratinized squamous metaplasia and development of cholesteatoma have never yet been observed, not even in Prussak's space where the many small lateral malleolar folds are to be found

In a much different way the mucosal folds play a decisive role in determining the direction of growth and the spread of the epitympanal cholesteatomata, still small in their early stage Initially the development of an anterior or posterior, a medial or lateral cholesteatoma depends upon the location of the mucosal folds a classification being of great importance to the otosurgeon

The stroma of the folds is almost always infiltrated by inflammatory cells, mainly lymphocytes, histiocytes and plasma cells. It is in the stroma that the most significant morphological alterations occur: rich vascularisation, newly formed gland-like structures, haemorrhage, fibrolysis, hyaline degeneration and calcium deposits as well as fatty degeneration and formation of cholesterol granuloma.

Often these folds undergo such marked fibrotic changes and grow so closely adherent to the surrounding mucosa of the walls and the ossicles that they can hardly be separated from one another. This rough polstering encompasses the ossicles until total fixation occurs, resulting in an adhesive process or a tympanosclerosis. Nevertheless, the function of the Eustachian tube mostly remains totally intact or is only slightly impaired and the mucosa of the meso-hypotympanum appears to be clinically normal.

In due course of time, however, the previously massively infiltrated and fibrotic folds in the region of the tympanic diaphragm will cicatrize and be retracted. This causes an interruption of air flow and drainage first of all, the retraction of Shrapnell's membrane to be followed. Later on, if the well developed interossicular folds between the neck of the malleus, long process of the incus and stapes are involved in this process, we observe atrophy and retraction of the posterior superior quadrant of the pars tensa also, her*mation and development of cholesteatoma* while the tube remains patent. In absence of ventilation and especially of drainage via the tympanic diaphragm accompanied by increasing replenishments of secretion we may encounter the appearence of so-called chronic mastoiditis. This is the effect of the main *pathological process in the epitympanum* mostly in the tympanic diaphragm and not the cause of the ear inflammation.

It is not my intention for the moment to list any conclusions regarding the relationship between these morphological alterations in the epitympanic mucosa and its system of folds, and the successful outcome of audiological surgical results. However, we are sure that without wide exposure of the main focus of *disease in the epitympanum, including its ana*tomically and topographically as well as physiologically important structures, without restoration of ventilation and drainage, surgery in the ear cannot ensure a lasting success. When all of the above-mentioned aspects are taken into consideration we will be able to preserve the ossicular chain in a large number of surgical interventions, to restore hearing to an almost normal degree, and most of all we will provide reairisation of the retrotympanal spaces, which can also be clearly noted in the X-ray pictures, as demonstrated in this last case.

RÉSUMÉ

L'origine et le developpement des replis muqueuses ont été explores. Comme une veritable suite de la muqueuse respiratoire de l'oreille moyenne elles se dételent entre les murs de la cavite et son contenu, sont riches aux vaisseaux et fibres nerveux et avec ces epithels respiratoires actifs et efficaces aux deux cotés elles agrandissent aussi la surface active de la muqueuse. Ces replis sont un facteur tres important pour le complexe de la défence de l'oreille moyenne. Hors de cette situation anatomique et topographique avec les conséquences pathophysiologiques on peut dériver une mode de l'origine des situations cliniques comme aussi une hypothese sur les troubles de l'aeration de la cavité avec une trompe d'Eustache bien fonctionnante.

ZUSAMMENFASSUNG

Die Entstehung und Entwicklung von Schleimhautfalten wurden erforscht. Sie sind als wichtiger Faktor in den Abwehrkomplex des Mittelohrsystems inkorporiert. Aus der anatomisch-topographischen Situation dieser Schleimhautduplikaturen mit ihren pathophysiologischen Folgen lasst sich der Entstehungsmodus der klinischen Befunde bei den verschiedenen Formen der Mittelohrerkrankungen und eine Hypothese uber die Beluftungsstorung der Paukenhöhle bei offener funktionsfahiger Tube ableiten.

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UNE EXPLICATION BIOCHIMIQUE ET CYTOLOGIQUE DE L'OTOSPONGIOSE COCHLÉAIRE

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Abstract Une étude portant sur 250 cas d otospongiose a démontré qu un teneur élevée en Trypsine dans la périlymphe des malades s accompagne dans 90% des cas d'une évolution vers une surdité cochléaire A l'inverse une teneur élevée en antitrypsine s accompagne d une absence d atteinte cochléaire dans une proportion de cas comparables

Nous avons précédemment démontre qu'il existait une correlation statistique precise entre l'activite hydrolytique de la périlymphe et l'evolution de l otospongiose vers une surdité de formule cochléaire (Chevance et al , 1972, Causse et al , 1972) Mais cette demonstration demeurait incomplete en ce sens qu'elle ne precisait pas le type d'enzyme en cause et n'avait qu une valeur qualitative puisque l etude se basait sur l intensite de la digestion des films photographiques par les prélevements de périlymphe

A partir de ces premiers resultats et compte-tenu des très faibles quantités de périlymphe qui peuvent etre recueillies lors de l intervention sans risque pour l audition de l'oreille opérée (3 a 5 μl) il est évident que s'imposait la nécessite de microdosages spécifiques Nous avons donc dosé en deux séries paralleles les valeurs de la trypsine et de l alpha-1-antitrypsine au niveau de la périlymphe de malades operés

MATERIEL ET METHODES

Nous avons utilisé pour doser la trypsine la méthode de B F Erlanger N Kokowsky et W Cohen qui utilise les propriétés de substrats chromogéniques de l'enzyme (Erlanger *et al* , *1961*)

Dans le détail on utilise 43 5 mg de chlorure de Benzoyl DL arginine *p* nitroanilide dilué dans 4 ml de diméthylformamide La dissolution s effectue en chauffant la solution aux environs de 70–80° Après dissolution on complète à 100 ml avec un tampon Tris pH 8–0 1 M la solution finalement obtenue est à concentration de 10–3 M

On incube 2 h à température du laboratoire +25° 0 7 ml de substrat plus une quantité de périlymphe variant de 3 à 5 μl l'enregistrement spectrophotographique s'effectue à 410 nm Les valeurs trouvées sont comparées à une courbe étalon (d activité standardisée) d'une quantité connue de trypsine purifiée

Ainsi a condition d'incuber un peu plus longuement que classiquement, la methode permet de mesurer l activité trypsique d un échantillon recueilli au niveau d une seule oreille – c'est à dire 3 a 5 μl de perilymphe quantité dont une expérience portant actuellement sur plusieurs milliers de prélevements nous a montré qu'elle pouvait etre prelevée sans faire courir de risque à l'audition de l oreille opérée

Les dosages de l alpha 1 antitrypsine au niveau de la périlymphe furent effectués en utilisant la méthode classique d immunodiffusion radiale en agarose Des lames de verre pour examen microscopique parfaitement nettoyées sont enduites sur une face d agarose à 2% à 80° elles sont ensuite mises à l'étuve à 60 pendant 24 heures

Dans un tube à essai on fait fondre à 80° 1 5 ml d agarose à 1 5% Dans un autre tube 0 5 ml de tampon véronal pH 8 2 est mélangé avec 10 μl de sérum anti alpha 1 antitrypsine (Behring) On mélange les 2 solutions à 58–60° Chaque lame de verre est recouverte du mélange de telle façon que la couche résultante ait un mill

Tableau I *Trypsine*

Trypsine (μg/ml)	Nombre de Cas CO=0	CO−+	CO ++	Total 99	% des cas non evolutifs	% des cas evolutifs
2 a 4 5	38	9	1	48	79 ?	70 8
4 5 a 6	15	16	4			
6 5 à 8 5	0	1	8	51	79 4	70 6
[illegible] 6 a 13	0	0	7			

CO 0 0 599+0 033 CO=+ [illegible] 705±0 041 CO−++ 0 897±0 077
La signification des pourcentages $P<1‰$

metre d épaisseur ensuite des cupules d une contenance de 5 μl chacune sont creusées dans cette epaisseur on reserve une de ces cupules sur chaque lame a titre de temoin pour evaluer la diffusion radiale de 5 μl d une solution d'activité connue d alpha 1 antitrypsine L immunodiffusion est effectuee pendant 38 heures a 37° puis chaque lame est lavée durant 48 heures dans 3 bains success fs d eau physiologique Apres séchage on colore avec une solution de bleu de Coomasie pendant 24 heures

L activite connue des differents echantillons temoins d alpha 1 antitrypsine permet d etablir une courbe sur papier semi logarithmique les zones de diffusion sont mesurées avec une précision d un ½ mm

Les deux series de malades etudies sont tout à fait comparables a la fois en nombre de cas et par l homogeneite de leurs caracteres en effet nous avons dose l activite trypsique chez 99 malades et l activite alpha 1 antitrypsique chez 103 Tous etaient atteints d otospongiose cliniquement evidente et audiometriquement verifiee Comme dans nos études précedentes qui portaient sur d autres aspects biochimique de l otospongiose nous avons distingue 3 groupes de malades

Les parametres audiometriques par lesquels nous definissons 3 classes de surdite sont les suivants

(1) est consideree comme surdite rapidement evolutive *CO++* une surdite dont la perte auditive atteint ou depasse 20 dB en deux ans pour la conduction osseuse au niveau des 3 frequences conversationelles

(2) est consideree comme surdité evolutive une surdite dont la perte auditive se situe entre 10 et 15 dB dans les memes conditions de temps et de frequence que precedemment *CO=+*

(3) est consideree comme surdite non evolutive une surdite ou la perte auditive ne depasse pas 5 dB *CO–0*

Il convient de preciser que ces parametres audiometriques sont ceux que nous avons constamment utilises au cours de toutes nos etudes sur l otospongiose Ils sont rendus plus precis encore du fait qu ils sont etablis dans les memes conditions a 6 mois ou au plus un an d intervalle dans la meme unite d audiologie qui dispose d appareils etalonnés chaque mois En effet le délai pre operatoire de la grande majorite de ces malades varie entre deux et trois ans Il en resulte qu ils sont evidemment surveilles du point de vue audiometrique tous les six mois

Dans ces conditions la precision des resultats audiometriques obtenus est comparable a celle des méthodes biochimique et immunologiques utilisees pour les dosages enzymatiques

Nos résultats et leurs traitements statistiques peuvent se resumer dans les tableaux I et II

Il convient de noter que les malades ont ete choisis strictement au hasard des tableaux operatoires de chaque jour et que les dosages biologiques furent effectues sous numero sans que l on sache l evolution clinique de la maladie

Ainsi pour les 2 series de malades etudies les valeurs statistiques sont hautement significatives il éxiste en effet moins d une chance sur mille pour que nos resultats soient dus au hasard

Tableau II *Alpha-I-antitrypsine*

Alpha 1 antitrypsine (µg/ml)	Nombre de Cas			Total	% des cas non évolutifs	% des cas évolutifs
	CO=0	CO=+	CO=++			
0 043–0 029	36	1	0	37	97 3	2 7
0 026–0 016	4	37	1	66	9	91
0 015–0 004	2	3	19			

CO–0 1 54±0 4 CO=+ 1 34±0 04 CO=++ 0 88±0 066
$P<1\%$

DISCUSSION

Tout d'abord insistons sur le fait que ces deux séries de dosages forment les deux volets d'une seule recherche et que les résultats de chacun d eux déja fort demonstratifs isolement sont singulièrement reforces lorsque l'on considere l'ensemble

L'existence d'un équilibre trypsine antitrypsine au niveau de la périlymphe est ainsi demontre on peut en effet raissonablement penser que les cas non evolutifs offrent un équilibre non perturbé ou au moins voisin de la normale, celle ci serait donc dans la périlymphe 2 a 4 µl/ml de trypsine pour 0,040 a 0,030 µg/ml d alpha-1 antitrypsine La zone ou s'effectue la rupture d'equilibre et donc l evolution vers une surdite d oreille interne se [illegible] entre 6 et 8 µg/ml de trypsine et 0 020 0 015 µg/ml d antitrypsine Il s'agit donc la [illegible] valeurs assez précises pour lesquelles l evolution bascule brutalement puisqu'a l equilibre l évolution vers la surdite n'atteint que de 2 a 20% des cas et apres rupture les valeurs s inversent, 90 a 70% des cas évoluent vers une surdite cochléaire plus ou moins rapidement évolutive

Les valeurs de la trypsine (et a contrario) de l antitrypsine semblent bien constituer un index de la gravite de l evolution puisque à partir de 7 µg de trypsine *tous* les cas sont evolutifs et le sont d autant plus que la quantite d enzyme augmente il en est de meme lorsque la quantité d alpha 1-antitrypsine diminue au-dessous de 0 010

Jusqu'ici le mécanisme par lequel l otospongiose, maladie en apparence purement osseuse provoquait, dans une proportion variant de 50 à 75% suivant les statistiques[1] une surdité cochléaire plus ou moins rapidement évolutive n'avait pas reçu d'explication satisfaisante c'est à dire étayée par l'expérimentation Nous pouvons affirmer maintenant que ce mecanisme est de nature enzymatique et qu'il s'agit plus précisement de la rupture de l'equilibre trypsine/antitrypsine au sein des fluides de l oreille interne

Nous remercions le Dr Causse et Mlle J Bergès auxquels a travail appartient aussi

SUMMARY

In a study of 250 otosclerotic perilymphatic fluids it was found statistically that in the presence of developing inner ear deafness 90% of cases will demonstrate increased trypsin activity Conversely elevated antitrypsin levels are found in the absence of developing inner ear deafness

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[1] Dans nos statistiques la proportion des malades qui sur deux années présentent une évolution vers une atteinte cochléaire oscille autour de 50% dans une statistique antérieure portant sur dix années George Shambaugh Jr signalait une proportion de 75% Ce qui est parfaitement cohérent avec nos résultats étant donné la différence des laps de temps considérés

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SECRETORY OTITIS MEDIA

Comparison of Nasal and Aural Cytology

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Abstract Aural and nasal cytology is compared in a series of 105 patients with secretory otitis media (SOM) In aural smears the cellular picture consisted mainly of lymphocytes leukocytes and phagocytes but eosinophils and basophilic cells were absent In the nose the most frequent cells were leukocytes while lymphocytes were seldom seen in greater numbers Increased numbers of eosinophils were found in 20% of the patients IgE values in the serum of 69 patients and in 8 aural effusions were mostly within normal limits and SOM could not be considered to be a result of circulating IgE-dependent reaction Cytotoxic leukocyte test with food allergens to test this etiologic factor in SOM is at present in progress

The etiology of secretory otitis media (SOM) is still somewhat controversial due to the factors involved At present it is agreed the middle ear fluid of SOM patients is not nearly as often sterile as was earlier thought but infection caused by various agents can be demonstrated In a material of 102 ears with chronic effusion Liu et al (1975) found bacteria by staining in 77 fluids and bacteria were isolated from 53 samples (52%) Eleven different species were found Diphtheroids being the most frequent in mucoid *Staph epidermidis* in serous and *Haemophilus influenzae* in the leukocytic effusions Klein (1975) isolated mycoplasm in 34 out of 661 specimens (4 7%) and infection with RS virus in specimens in the period of epidemics Using increased titres as evidence virus infection could be diagnosed in 270 or 21 5%

Allergic factors in the etiology of SOM have been duly recognized but positive evidence such as found in infectious etiology is scarce This was discussed in a recent panel (Shambaugh, 1975) where Lecks (1975) reported the use of the radioimmunosorbent (RIST) test to determine the total IgE in the serum of 34 babies followed from birth to the age of 22 months Low IgE values of 0 to 2 units/ml should appear in the newborn period gradually rising to 20 units/ml Eleven of these children all showing IgE values of over 20 developed allergic manifestations but not SOM Adult values up to about 500 units/ml were generally reached at the age of 10 It was concluded that single quantitation of IgE adds little to support a diagnosis of allergy and the possibility remains that tissue response in SOM is mediated by other mechanisms than by IgE e g by cytotoxic reaction or by delayed cellular hypersensitivity Wilson (1975) pointed out that only the Eustachian tube mucosa may be reacting as the shock organ and consequently SOM patients need not show symptoms of allergic rhinitis In this panel discussion the estimations of allergic etiology ranged from 20 to 90%

At the recent symposium on middle ear effusions in Columbus Ohio Bryan & Bryan (1975) felt that in addition to the leukocytes whose behaviour is principally recorded in the cytotoxic test the lymphocytes and macrophages are also involved through the delayed hypersensitivity mechanism in the

Table I *Inflammatory cell pattern in effusion (157 ears)*

Cell type	None	A few	Moderate	Abundant
Neutrophils	24	33	53	47
Lymphocytes	4	18	91	44
Eosinophils	149	7	1	–
Basophils	151	6	–	–
Monocytes	34	97	25	1
Phagocytes	21	53	73	10
Giant phagocytes	143	13	1	–

development of SOM Clemis (1975) stressed the important part foods play in the etiology of SOM, and re-emphasized that the clinical data need not be related to IgE levels The cellular picture of middle ear fluid in SOM (Palva et al, 1975) showed large numbers of lymphocytes and polymorphonuclear leukocytes while only a few specimens had some eosinophilic and/or basophilic cells

In the present study we have correlated the findings of cytological examination of aural and nasal smears in order to see whether there is any agreement between these two sites in SOM according to clinical cytologic criteria of allergy In addition, some data on the IgE values are reported

MATERIAL AND METHODS

Satisfactory smears of the middle ear effusion were obtained from 157 ears in a material comprising 105 patients In 82 of these patients the nasal exfoliative cytology was studied simultaneously Staining and evaluation of both aural and nasal smears were the same

Table II *Inflammatory cell pattern in nasal secretion (82 patients)*

Cell type	None	A few	Moderate	Abundant
Neutrophils	4	14	29	35
Lymphocytes	42	35	5	–
Eosinophils	41	24	12	4
Basophils	80	2	–	–

Table III *Epithelial cell pattern in nasal secretion (82 patients)*

Cell type	None	A few	Moderate	Abundant
Columnar cells	1	–	4	77
Goblet cells	7	48	21	6
Viral forms (CCP)	43	27	11	1
Squamous cells	56	13	13	–
Metaplastic cells	82	–	–	–

as reported earlier (Palva et al, 1975) The radioimmunosorbent (RIST) test was used to determine the total IgE level in the serum of 69 patients, and in 8 aural effusions Nasal bacterial cultures were done in 95 patients, and aural cultures in 98 patients

RESULTS

The findings as to type and frequency of inflammatory cells in the aural smear are shown in Table I It appears that lymphocytes and neutrophils dominate the cellular picture, followed by phagocytes Eosinophils occurred in 8 and basophils in 6 samples, but only one smear showed eosinophils in numbers that could be considered increased

The inflammatory cell pattern in nasal smears is shown in Table II Here the neutrophils are the dominating cells, lymphocytes being absent even from more than half of the samples Eosinophilic cells were seen in increased numbers in 16 samples (20%) whereas basophils were seen only in 2 samples and their number was too small to be regarded as increased

Epithelial cells in the nasal smear consisted mainly of columnar cells and goblet cells Viral forms were seen in 39 samples (43%) (Table III)

The findings of the bacterial cultures of nasal and aural specimens are presented in Table IV The nasal samples were clearly more often positive than were the effusion specimens, and particularly pneumococci and haemophilus strains were more frequently

Table IV *Bacterial findings in nasal and aural cultures*

	Nasal secretion	Aural effusion
Normal flora or negative	32	54
Staph aureus	18	19
Pneumococci	22	5
Haemophilus	12	7
Others	4	13

present in the nose No correlation was found between the bacterial species cultured from the nose and the ear of the same patient

Considering the age normative IgE values normal levels were found in 59 and raised values in 10 patients In 5 of them there were increased numbers of eosinophils in the nasal smear while in 5 the nasal cytology was normal Six aural specimens had normal and two raised levels of IgE (Table V)

DISCUSSION

It is evident that aural cytology does not offer any help in the allergic etiological problems of secretory otitis media when applied in its conventional form numbers of eosinophils and ast cells indicating allergy The main cellular ments in the thick fluids are neutrophils mphocytes and phagocytes Occurrence of hese cells may indicate infectious etiology but on the other hand the difference between the inflammatory cellular populations of the aural and nasal smears is rather striking The possibility remains as was suggested by Bryan & Bryan (1975) that lymphocytes and macrophages are related to the immunosystem defect and thus involved in the persistence of the process in the middle ear

The nasal smears in SOM seem to show distinct signs of allergic response in the form of eosinophilic cells Their frequency, 20% is somewhat higher than the frequency of allergic manifestations in the population (Engstrom et al , 1960, Alanko 1970) and it might be interpreted to mean that SOM is partly related to the allergic background of these children So far, however, there are no reports on the occurrence of secretion eosinophils in nasal smears in a random selection of the population nor on how often secretion eosinophils really are indicative of manifest clinical allergy Considering that the values of serum IgE are mostly normal in the present study it can only be concluded that SOM does not seem to be related to a circulating IgE-dependent form of allergic reaction

As was discussed in the introduction other not IgE dependent systems could be involved particularly in the induced cytotoxic reactions This could be tested by using the refined cytotoxic test of the Bryans in a random material of SOM patients Altered reactivity to foods manifested in immobilized and/or killed leu

Table V *IgE level in serum of 69 patients with secretory otitis media*

	IgE level (U/ml)					
	Normal	Raised				
Age of patient	0–13	0–100	3–150	3–210	5–390	>400
0–5 months						
1/2– 2 years		13	2			
3– 6 years	3	17	5	1	2	2
7– 9 years	1	5	3	2	2	1
10–12 years		5			2	
13–15 years		1				
>15 years		2				
Total	4	43	10	3	6	3

kocytes brought in contact with food allergens, would still agree with the established infection etiology. This toxic reaction could release the slow reacting substance from the damaged leukocytes and lower the quality of the immunosystem so much as to allow an infection to develop. Again, the chronicity and persistence of the disease in these cases could be due to continued reaction to these nonrecognized allergens.

We have now started a systematic study of our SOM patients using the cytotoxic test and in 20 analyses made so far, moderate or often marked reactions have been found as regards some of the foods. Cow's milk and wheat seem to be the most frequent foods to react.

The proof of the cytotoxic test result can only be found in the recovery of the SOM patient. When the offending food is removed and an adequate rotation diet arranged, the middle ear effusion should disappear quite quickly, and insertion of grommets should not be necessary. Only a limited number of observations are available at present (Wilson 1975) but with the increased use of the cytotoxic test many unsettled problems may be better understood.

RÉSUMÉ

La composition cellulaire de la sécrétion nasale et aurale est analysée avec l'intérêt special aux cellules éosinophiles et basophiles. La relation des immunoglobulines dans le serum et dans la sécrétion de l'oreille moyenne est discutee specialement celle de l'IgE.

ZUSAMMENFASSUNG

Ohren und Nasenzytologie wurde in einem Material von 105 Patienten mit sekretorischer Otitis media (SOM) korreliert. Lymphozyten, Leukozyten und Phagozyten dominierten das Zellbild der Mittelohrflussigkeit, wahrend Eosinophile und Basophile abwesend waren. Die haufigsten Zellen in dem nasalen Abstrich waren Leukozyten und Lymphozyten kamen nur selten in großerer Menge vor. In 20 Prozent der Patienten war die Anzahl der Sekreteosinophile erhoht. Das IgE Niveau im Serum von 69 Patienten und in 8 Mittelohreffusionen war meistens normal und der Schlußsatz wurde gezogen, daß SOM nicht zu den Reaktionstypen gehort, wo zirkulierende IgE Reagine entstehen. Eine Untersuchung mit den zytotoxischen Leukozyttest mit Nahrungsmittelallergenen ist jetzt unternommen worden, um diesen etiologischen Faktor in SOM festzustellen.

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DISCUSSION

I Friedmann I was interested in your negative findings of eosinophils in the aural secretion. But you then stipulated that allergic mechanisms play an important role in the causation of the glue-ear syndrome. I believe that the pathological substrate of the glue-ear syndrome is the secretory mucosa of the middle ear as and parcel of chronic otitis media.

T Palva (Reply) to Mr *Friedmann*. It is really a fact that the ear secretions in glue ears are devoid of those cellular elements, eosinophils and basophils, which we classically regard as indicators of an allergic response. We are in complete agreement that the secretory mucosa of the middle ear has primary importance in this syndrome of the glue ear. However, in addition to infection allergic

phenomena not associated with eosinophils or mast cells may play a significant role I am referring to delayed hypersensitivity reaction which is mediated by sensitized lymphocytes and in which the polymorphonuclears and monocytes act as indicator cells It seems that food antigens may be the cause of lymphocyte sensitization in many cases and the tissue reactions are due to the secreted lymphokines in the form of MIF and cytotoxic factor which cause the polymorphonuclears to be totally destroyed when brought in contact with food antigens This liberates many active substances including SRA A which may be one cause of the marked mucosal changes These reactions are not eosinophil or mast cell-dependent and from this fact derives my belief of the importance of allergic factors in secretory otitis media so far not generally recognized

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ULTRASTRUCTURAL STUDY OF TASTE BUDS AT REST AND AFTER STIMULATION AND COMPARATIVE STUDY BETWEEN TYPE III CELL AND MERKEL CELLS

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Abstract Changes in the pore region of the cell membrane have been studied in order to learn if this region plays an active part in the preneural phase of taste We have observed though not consistently that the pore size is variable and that their contents are not constantly amorphous or homogeneous The results are not so significant however as to allow of the formulation of any firm conclusions The similarity between type III cells and Merkel cells (cells of the tactile system) was surprising The probable role of the type III cell in taste is discussed

In 1954 Beidler proposed a receptor theory of taste cell stimulation which was based on the physical binding of the stimulus molecules to specific sites on receptor molecules presumably located in the membranes of the taste cells A consideration of the kinds of molecules that have been found in cell membranes suggests that proteins are the most likely candidates for taste receptor molecules The term receptor sites was proposed by Beidler (1971) to indicate the particular site on the receptor molecule with which a given stimulus ion or molecule can interact

According to Harper (1972) the recent renewal of interest in the possible relation between taste qualities and molecular structure is a reflection of the successes achieved in applying the principles of molecular biology to other problems However some conceptual reservations should be expressed Although the initial processes of stimulation must depend upon some form of molecular interaction with the cells of the peripheral sense organ it is hardly reasonable to expect a complete explanation of quality in terms of biochemical action at these levels Nevertheless there are many facts in evidence at present that allow a consideration of the significance of the biochemical events in the early phases of the taste process

We may consider that the first event in the taste process is some kind of biochemical reaction between the stimuli and the surface of the taste receptor It is therefore the composition and structure of the cell membrane and in particular of that portion of it on the surface exposed to the stimuli which determines the nature of the inception in the taste response (Tamar 1972)

These facts have focused the interest of the researchers on the cell membrane The microvilli probably constitute the only part of the cell that can come in contact with the stimuli In fact according to Murray (1973) the taste cells in the bud are tightly sealed together beneath their necks Thus only the microvilli stay in the taste pit

The cell membrane according to the Danielli model appears to be basically composed of two inner layers of radially oriented phospholipid molecules and two surface layers of protein molecules tangentially orientated The hydrophobic hydrocarbon ends of the phos

must penetrate through the pore of the taste bud At the conference held in Bethesda in 1969 on The Molecular Basis of Taste and its Disorders great emphasis was put on the taste pore as an important element in the pre neural events of the process, regulating the flux of sapid substances

Graziadei (1969) stated that so far, little attention has been paid to the study of its function The pore is approximately 1 micron in diameter at the lingual surface, however, *in vitro* observation of its dynamics through the stereomicroscope does not allow a thorough estimate of its variation in size Observations with the scanning electronmicroscope, however, suggest that variations in taste pore size do occur Sometimes the pore is closed, whereas in other instances the conical top of the bud protrudes and the cavity of the pore can be clearly visualized The pore opening is sometimes obliterated by mucous, while at other times it is completely devoid of any material and appears empty Analysis of several electron micrographs has demonstrated that the pore opening varies considerably in diameter and these observations support the possibility that the pore may act as active diaphragm as was suggested by in (1969)

According to Henkin (1969) it is this initial event in the taste process the penetration of tastant through the taste pore and its membrane that he hypothesizes as the part of the process which is influenced by thiol and metal concentrations Protein molecules lining the pores of the taste bud membrane are regulated by multiple equilibria involving thiols and metals These in turn control the diameter and permeability of the pores and their membranes He designates the protein molecules that regulate the amount of tastant that passes through the pore of the taste bud membrane per unit of time as the *gatekeeper protein* To change the effective diameter of the small pores of the membrane we need invoke only small conformational changes of the order of magnitude of 10 Å in several proteins To make an appreciable change in the effective diameter of the larger taste bud pore would require a more drastic conformational change such as is produced by protein aggregation or unfolding

The study of the taste pores from the tongues of hypogeusic D-penicillamine treated rats by scanning electron microscopy, suggests that many more taste pores may be closed than in the case in untreated animals

There is one disease, *Familial Dysautonomia* or *Riley Day syndrome* in which papillae and taste buds are absent in man (Farbman 1971) *Familial Dysautonomia* is a rare inherited disease characterized by many neurological symptoms, e g posturapostural hypotension, refractoriness to pain, absence of tears and several other symptoms, including the inability to taste In the tongue of these patients the only neural structures found, are unmyelinated nerve fibres that do not terminate in any specialized epithelial structure There are no taste buds

In these patients the subcutaneous or intravenous administration of methacholine produced tears, and taste appear This fact suggests that the taste bud per se may be unnecessary for the mediation of the taste response Parenteral methacholine would be associated with a general increase in membrane permeability, including that of the lingual surface This effect would allow the taste stimulus to reach the unmyelinated nerve fibres of the tongue, the only element competent to sense taste Thus taste is more close to chemical sense

A major question raised by these observations appears to be what role, if any, do taste buds play in the taste process This question has never been adequately answered One suggestion, based on these observations is that taste buds play the role of chemical sieves Yet we know that the pit region of the taste bud is isolated from the hypothetical free nerve endings

We decided to study the pit region of the taste bud in order to learn if any change oc

Fig 1 Normal taste bud without any stimulation Semi thin section toluidine blue staining ×1000

curred after stimulation We focused our attention on four different aspects the pore's diameter, the amorphous substance of the pit, the type III cells and their synapses The first two points are the specific ones, the secondary ones are considered of interest in spite of not being structures of the pit region The type III cell according to Murray (1971–73) is the only cell with real synapses, and so probably the only functional element Both cell and synapses are studied

The dense and amorphous substance of the pit is of enormous interest because in it would be located the hypothetical proteins involved in the biochemical regulation of taste This substance has been characterized by Scalzi (1967) as neutral mucopolysaccharides although Baradi & Bourne (1953) could not find mucoid here and Erbengi & Ferner (1964) did not show positive PAS for this region

By studying type III cells we realised that these elements are very similar in their morphology to Merkel cells These cells are not well known, yet are very widespread They have been found in the tactile disc of the hairs, in the Grandry corpuscle, in the dermis (Winkelmann & Breathnach, 1973) As a very interesting aspect for our study, they have also been found in the hard palate and gum mucosa (Hashimoto, 1972, Winkelmann, 1960, Kutuzov & Sicher, 1952, Taylor et al , 1964) We decided to include a comparative study of both elements in an effort to explain some physiological facts This matter has never been studied before

MATERIAL AND METHOD

Twenty four adult rabbits were used 16 for electron microscopy and 8 for optic microscopy study Moreover we used one specimen of pig snout to study the Merkel cells

Six rabbits were sacrificed without stimulation, 4 after 48 hours fasting and the others after various kinds of stimulation

sweet (saccharose 30%) 2 animals
salty (sodium chloride 25%) 3 animals
sour (citric acid 100%) 2 animals
bitter at (quinine sulph 50%) 2 animals
normal feeding 5 animals

All animals were decapitated 3 min after the stimulation In order to stimulate the

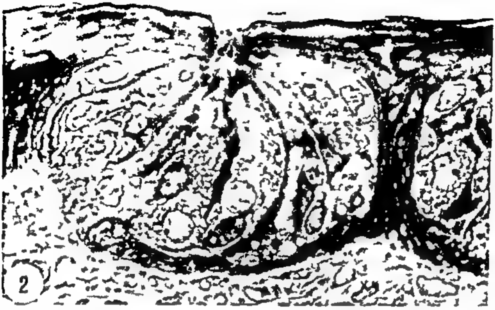


Fig 2 Taste bud after stimulation with sodium chloride showing a slight opening of the pore and less amorphous substance Semithin section toluidine blue staining ×1000

tongues we poured 2 cc of the solution into the mouths repeating it a minute later

The tongues were removed easily and we obtain the foliate papillae

The specimens for electron microscopy were cut in a prismatic way keeping the ...ation reference 2 5% glutaraldehyde 1 1% osmium tetroxide were used for fixa...on with a common buffer system (phosphate solution according to Millonig pH 7 4) Acetone was used for dehydration and the specimens were embedded by Spurr s method

Ultrathin sections and thicker (0 5 or 1 μm) sections were cut on an LKB Ultratome with glass knives at 45° They were examined in the JEM 100B electron microscope

We used for optical examination hematoxylin–eosin staining Mallory s method and semithin sections These sections were prepared from the total surface of the block for electron microscopy and cut in 0 5 or 1 μm sections 1% toluidine blue in borax was used as stain

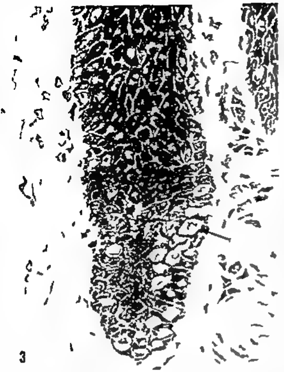
RESULTS

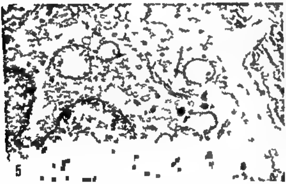
In the non stimulated specimens we could see the normal morphology of the taste buds (Fig 1) both with optical and electron microscopy The pores were quite closed and amorphous substances showed a uniform appearance (Pores are normally closed and the substance in a homogeneous state)

In the animals submitted to different kinds of stimulus we realise that some pores are wider and there is less amorphous substance which is disposed in an irregular and non homogeneous way (Fig 2) This arrangement was also seen with electron microscopy The phenomenon was quite similar for all stimulation conditions perhaps more remarkable with salt We could not detect any changes in microvilli dense granules of cell type I or III and synapses

These results however are not so remarkable or frequent as to state categorically that any real change has occurred In some animals they were lacking completely and in most we could see them inconstantly and only in some buds

As already mentioned we realise that type III cells are very similar in their morphology to the Merkel cells We therefore decided to extend our work to include a study of this





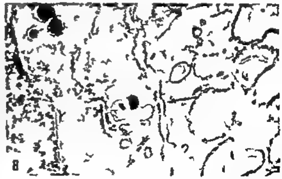


Fig. 3. Pig's snout Merkel cells (*arrow*). Semithin section toluidine blue staining. ×1000.

Fig. 4. Merkel's cell (*M*) with its nerve ending (*N*). Lobulated nucleus and dense granules close to the nerve ending. ×55000.

Fig. 5. Golgi apparatus of a Merkel's cell (*arrow*) showing dense granules. ×1 0000.

Fig. 6. An unmyelinated nerve entering a Merkel cell (*arrow*). ×50000.

Fig. 7. Synapse-like structure with Flamentous matter (*arrow*) and clusters of dense vesicles close in a Merkel cell. ×180000.

Fig. 8. A synaptic-like structure in a Merkel cell with dense vesicles that have lost their contents. ×180000.

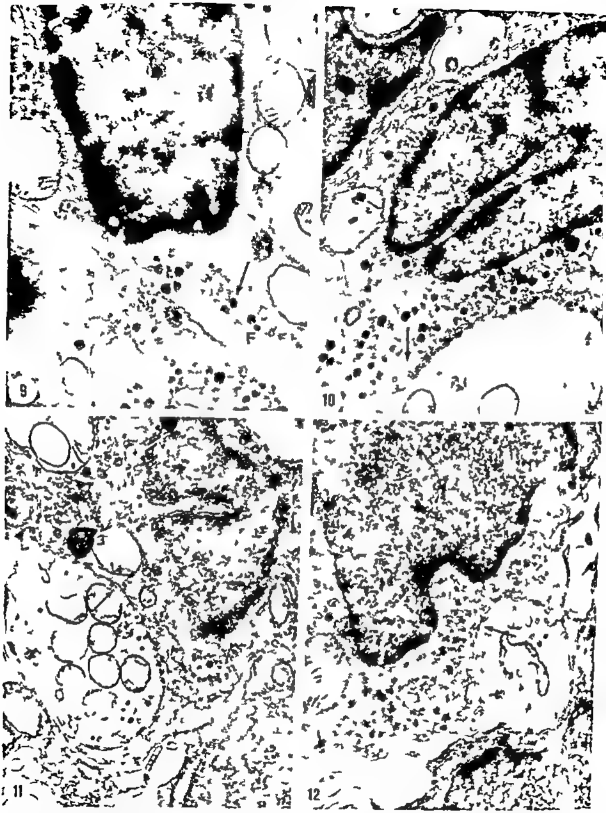


Fig 9 Type III cell of the rabbit Dense ves cle n ts neural pole (*arrow*) ×70000

Fig 10 Type III cell with dense and l ght vesicles (*arr w*) ×70000

Fig 11 Synapse between type III cell and nerve end ng (*arrow*) ×75000

Fig 12 Synapse-l ke structure between type III cell and nerve end ng (*arrow*) There are dense ves cles some of them without con ents ×80000

interesting aspect that has never previously been considered

A specimen of pig's snout was used because in this animal Merkel cells are more evident than in others

In Fig 3 we can see these cells in semithin section Merkel cells are rounded (Fig 4) with lobulated nucleus They are situated in the epidermal basal layer and they have in their cytoplasm specific granules of 700–1800 Å, they are dense and are situated far from the Golgi apparatus (Fig 5) Here it is possible to see some granules in growth stage

These cells are always next to nerve fibres (Fig 4) and the dense cytoplasmatic granules are situated in proximity to the nerve In Fig 6 we see the entry of an unmyelinated nerve into the cell

We cannot see synaptic vesicles but we observe dense vesicles close to the nerve endings In Fig 7 there is a synapse like structure with membrane reinforcement A synapse like structure with dense vesicles that have lost their contents ■ also seen (Fig 8)

Type III cells of the taste bud are quite similar to Merkel cells (Fig 9) They have dense vesicles in the neural celular pole but also light vesicles in real synaptic contact with unmyelinated endings (Figs 10, 11)

In a similar way to Merkel cells, synaptoids were seen with an accumulation of dense vesicles (Fig 12) Some of them have an empty appearance

DISCUSSION

As stated earlier, we cannot draw any definite conclusions from our observation about the opening of the pore after stimulation and the changes in the amorphous substance Graziadei (1969), Henkin & Bradley (1969) have considered that an active function in the pore does exist, as we also mentioned briefly earlier In our material some changes in the pit region were occasionally evident, but not frequently We wondered if the method of stimulation was inappropriate In order to confirm this fact, we intend to continue our work, with a more selective method of stimulation On the other hand, we must consider the possibility that a real specificity of the taste bud exists for the different sapid substances In the light of previous electrophysiological work, this is not likely

The resemblance between the type III cell and the Merkel cell, opens up ■ new field of research It is debatable whether the possible changes in the medium containing the microvilli can act by cellular deformation, as occurs in intradermal cells with tactile endings

However, the fact that the sensory vesicles of type III cells are found in the neural pole of the cell prompts the consider that they more probably play some part in the nerve impulse transmission The type I cells, or dark cells, also have dense vesicles but in the apical pole, close to the pit region, and it has been suggested that they are related with the amorphous substance of the pit

According to Farbman (1971), at approximately day 15 of intrauterine development, the epithelial basal cells of the bud contain clusters of vesicles within its cytoplasm, adjacent to nerve processes which are quite similar both to vesicles of Merkel cells and to type III cells of the taste bud These vesicles very soon disappear It has been surmised that they possess a trophic factor to attract specific nerves to grow into these fields In type III cells these elements remain throughout life, probably as a reinforcement of its nerve contact

In the system of Merkel cells there is a variation in the size of its granules (Saxod, 1970) as in type III cells In Grandry corpuscles, Andersen & Naftad (1968) have seen synaptoid structures Our observations of synapse-like structures in Merkel cells are in agreement with the works of Halata (1970) and Soe-Yeh Chen et al (1972) nucleus dense vesicles are present close to the synaptic zone It is probable that they act as a transfer element discharge by cellular deformation

A double synaptic mechanism is present in type III cells according to the fact that they possess vesicles having a light nucleus and a dense one, it is reasonable that they may work in a similar way as they do in Merkel cells

According to Breathnach (1971) and Hashimoto (1972) Merkel cells probably originate in the neural crest moving with nerve fibres Type III cells may be of similar origin Since the last century, various authors have discussed the origin of taste buds The role of the nerve has been the object of attention and many studies have been made without reaching agreement Probably the specific nerve induces the formation of the bud in the epithelium of the tongue

We must point out that the type III cell is the only element with synaptic equipment in the bud We may surmise that they constitute a real neural element as is the case in all neurosensory elements The activity of the dense vesicles is manifest, we could see some of them devoid of contents

Probably other neural crest elements glands medulla, suprarenal cells or endocrine elements of the alimentary canal etc both Merkel cell and type III cells would be neuroendocrine elements capable of supplying amoamines or other substance

RÉSUMÉ

Une etude ultrastructurelle des boutons gustatifs du lapin en repos et après une stimulation On discute les resultats et on les met en rapport de façon spéciale avec la theorie moleculaire de la regulation de l acuite gustative

ZUSAMMENFASSUNG

Man hat die Veranderungen in der Zone des Geschmacksporus nach der Anregung studiert und man hat auch versucht ob diese Zone eine aktive Rolle bei der praneuralen Geschmacksentwicklungsstufe spielt Einen großeren Durchmesser der Poren obwohl unbestandig sowie eine Verminderung der formlosen Substanz welche auch ziemlich zerstreuter aussieht wurde beobachtet Die Ergebnisse sind nicht beständig genug um entscheidende Schlußfolgerungen zu erlangen Die große Ahnlichkeit zwischen den Typ-III Zellen der Geschmacksknospe und den Merkel Zellen (d h Zellen des Tast Systems) war uberraschend Die mogliche Rolle der Typ-III Zellen beim Geschmackssinn wird diskutiert

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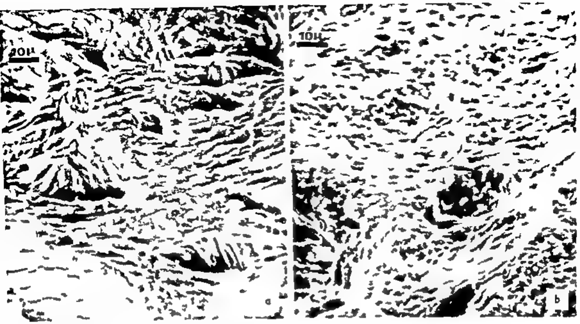

Fig 1 (*a*) Normal crus of the stapes on the outs de ×1000 (*b*) Footplate Tympanal surface ×1000

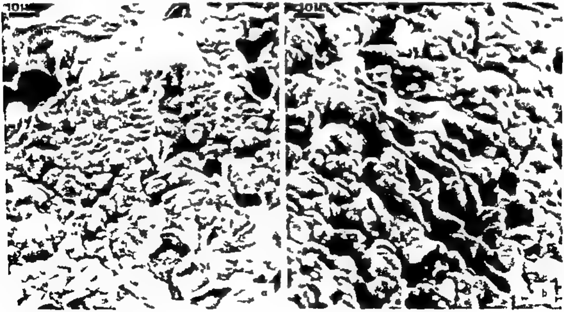

Fig 2 (*a*) Footplate vest bular surface ×1000 (*b*) Rim of the footplate ×1000

footplate of normal and otosclerotic stapes could be examined If only fragments of the otosclerotic footplate remained the vestibu lar sides were examined However a series of experiments were performed to compare the tympanal and vestibular sides of normal foot plates

The microscope used was a Cambridge

Fig 3 a–b Fractured footplate The junction of the two bony structures (*a*) ×100 (*b*) ×300

Scanning Electron microscope MK II, operating at 30 kV The utmost resolution that can be obtained in this instrument is of the order of 200 Å This corresponds to a theoretical magnification of more than ×20 000 All [illegible] shown in this paper were recorded in [illegible] so-called secondary mode which means [illegible] all secondary electrons (backscattered primaries and true secondaries) were detected *Electron beam damage proved to be negligible* and was observed on one sample only

RESULTS

Normal human stapes

All pictures of the normal human stapes have been obtained from simultaneously treated fragments of one specimen The crura show bundles of parallel fibres, plaited into a smooth surface on the outside and a rougher surface on the inside The structure of the tympanal surface of the footplate is very similar to that of the inner side of the crura (Fig 1) It differs markedly from the vestibular surface of the footplate (Fig 2) This has a more irregular aspect, as if centres of formation of very dense bone had fused together, leaving many irregular crypts between the lumps The bony masses themselves show many very fine pores not present on the tympanal side

The latter bone structure, but without the fine pores, extends as a peripheral layer on the rim of the footplate On the tympanal side of this rim the junction of the two bony *structures is clearly visible after fracturing* the footplate (Fig 3) This fracture allows one to distinguish clearly the two layers and more especially the many crypts in the vestibular part of the footplate

Otosclerotic stapes

In the present study the otosclerotic nature of human stapes was established on a clinical basis and on their appearance at operation since due to the trypsin treatment *it is not* possible to perform control light microscopy before or after the S E M experiments

In the first stage only the anterior part of the stapes and the connecting part of the crus anterior become spongy (Fig 4) The oto-

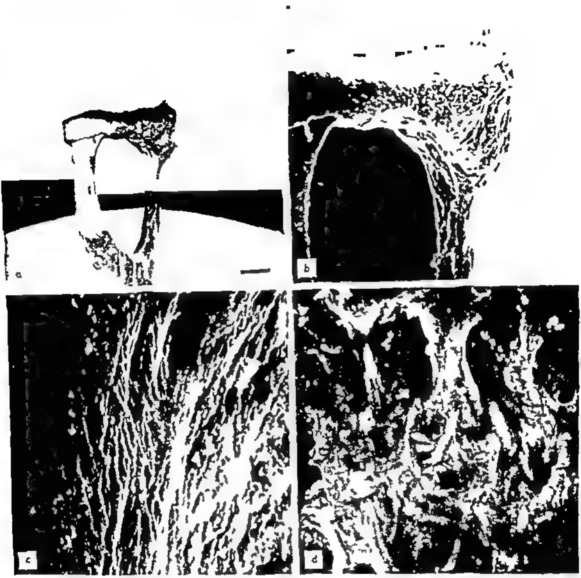

Fig 4 a–d Otosclerotic stapes (Stage I) *(a)* ×19 *(b)* ×48 *(c)* crus posterior ×1100 *(d)* crus anterior ×1100

sclerotic crus anterior loses its lamellar structure and becomes more amorphous and spongy the crus posterior remains unaffected in this stage and hardly differs from a normal crus

In the middle of the footplate in the first stage active otosclerotic bone is present next to normal regularly arranged bone trabeculae in connection with spongiform bone (Fig 5) In stage two the footplate is totally involved in the otosclerotic process but the web like bone neoformation is more pronounced This weblike structure in between amorphous structures is nearly complete in the third and fourth stages (Fig 6)

Stapes footplate in osteopsatyrosis

S E M observations were also made on a sizeable piece of the footplate of a far advanced stage (third or fourth) of osteopsatyrosis In

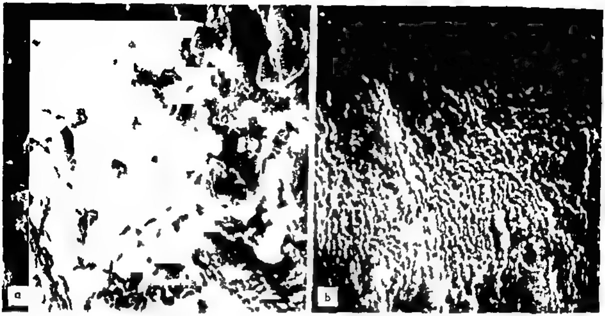

Fig 5 a b Otosclerotic stapes (Stage I) *(a)* ×950 *(b)* ×10 000

contrast to the case of otosclerosis we found only a more amorphous structure of the bone without any web like structure (Fig 7)

Although this observation remains to be confirmed in further cases the present three dimensional observations seem to indicate that in osteopsathyrosis the spongy bone is even more amorphous and that the irregularly arranged web like bone structure is absent

Fractured stapes footplate in rabbit

The left footplates of a series of rabbits were compared with the right footplate fractured with a blunt needle 5 months earlier The normal structure of the footplate in rabbits differs from the normal human footplate only by a finer regular arrangement of mineralized collagen bundles (Fig 8) We were surprised however to find that after 5 months the broken footplate was thicker than normal and very firmly fixed in the oval window S E M after a trypsin treatment identical to that used for the otosclerotic footplates showed only a poorly defined spongy destruction however the normal mineralized collagen bundle pattern was replaced by a web like structure of bundles similar to the one observed in the last three stages of otosclerosis

DISCUSSION

Normal footplate

Thorough examination of the different constituents of the normal human stapes brings us to the conclusion that at least the structure of the vestibular part and of the peripheral layer of the rim of the footplate differ from that of the tympanal part and of the crura Although this observation does not allow us to conclude that both structures have a different embryonic origin it does seem to favour this view which was originally proposed by Bast & Anson (1949)

As far as *otosclerosis* is concerned our observations are in agreement with the results of *Chevance* et al (1973) although we have used unfixed material Thus we found in the first stage of otosclerosis formation of spongious bone in the otosclerotic foci In the following stages we were able to establish the existence of an irregular structure probably due to combined destruction and formation of new bone

A comparable bone pattern was observed as

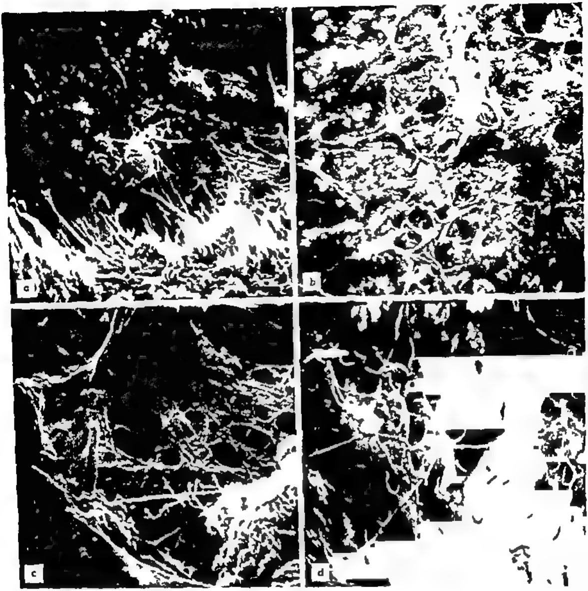

Fig. 6a–d. Otosclerotic stapes. (*a*) Stage 1. ×10000. (*b*) Stage 2. ×1000. (*c*) Stage 3. ×10000. (*d*) Stage 4. ×4400.

the result of the healing process of *fractured footplates* of rabbits.

In one case of advanced *osteopsathyrosis* the occurrence of the spongious bone was not accompanied by new formation of bone. This observation demands confirmation, of course.

One line of our further research will be to examine other early stages of otosclerosis to make clear which part of the footplate, the tympanal or the vestibular one, is the initial site of the pathological process.

RÉSUMÉ

La microscopie électronique à balayage nous a permis d'étudier la structure tridimentionelle de l'étrier normal et dans les différents stades d'otosclérose. Un cas d'ostéopsatyrose est détaillé. La formation d'un callus osseux après fracture expérimentale est comparée avec l'otosclérose.

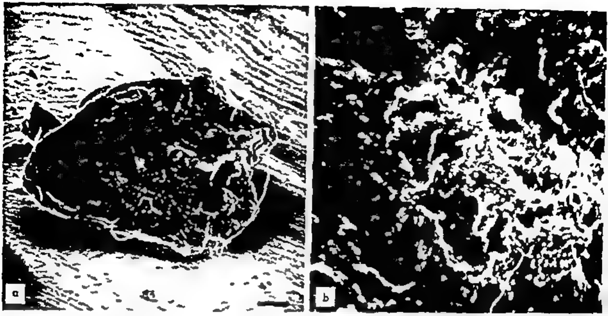

Fig 7 a–b Osteopsatyrosis stapes (*a*) ×40 (*b*) ×200

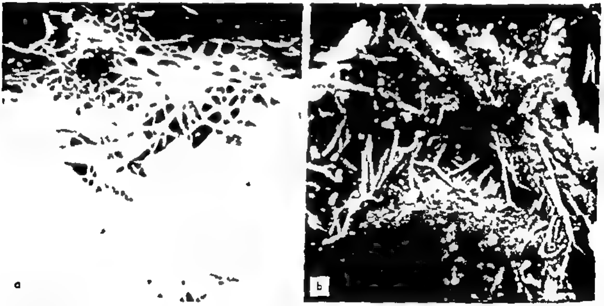

Fig 8 a–b Rabbit stapes (*a*) Normal ×13000 (*b*) *Fractured* ×13000

ZUSAMMENFASSUNG

Mit Gebrauch der E S M haben wir die dreidimensionelle *Struktur des Knochens normaler Steigbugel* studiert ebenso die Strukturen der verschiedenen Stadia der Otosklerose Ein Fall von Osteopsatyrosis wird ebenfalls besprochen Die Callusbildung bei experimenteller Fraktur des Steigbugels wird verglichen mit Otosklerose

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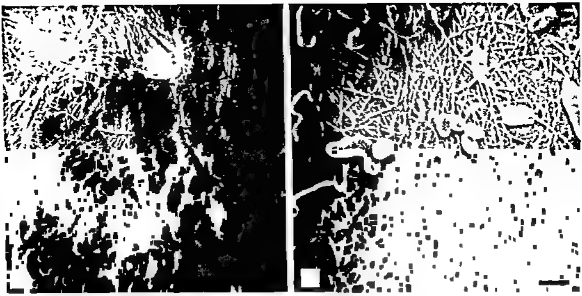

Fig 9 a–b Normal human stapes (*a*) Vestibular side ×10000, (*b*) Tympanal side, ×10000

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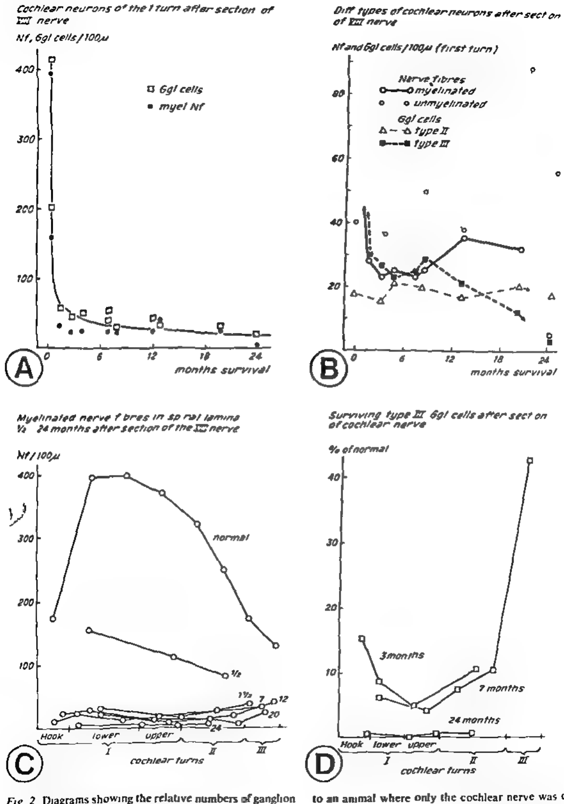


Fig 2 Diagrams showing the relative numbers of ganglion cells and nerve fibres following transection of the VIII nerve related to the survival time and to the cochlear turns In (B) the values of 24 months survival are related to an animal where only the cochlear nerve was cut and therefore only very few myelinated fibres are found (see Fig 1 A)

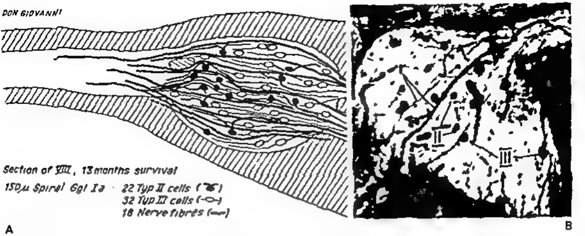
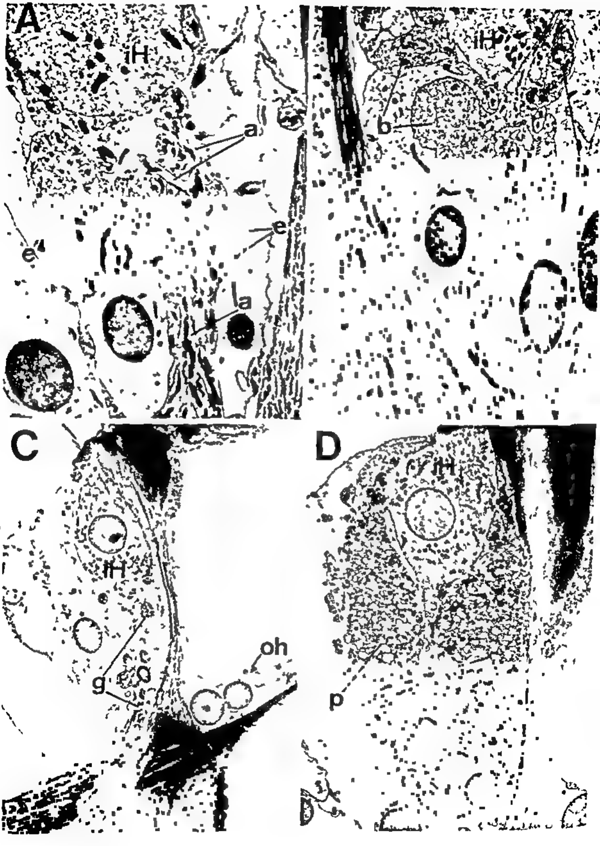


Fig 3 (A) Reconstruction of a series of five 30 µm thick sections of the osseous spiral lamina 30 months after sectioning of the VIII nerve Showing the surviving type II and III cells and the directly passing nerve fibres (B) One 30 µm thick section through the osseous spiral lamina of the upper basal turn of a cat 20 months after transection of the VIII nerve With interference contrast the surviving ganglion cells of type II and III (II & III) can be recognized classified and counted Directly passing regenerating myelinated large and small nerve fibres (*r*) are clearly seen

the normal exclusively radial distribution (Fig 4 A, 4 B) Many fibres become unusually large and form buds containing large numbers of small mitochondria as an expression of enhanced metabolism As time goes on, more and more elements will show signs of degeneration simultaneously with proliferative tendencies in the form of necrobiosis Degenerative processes will finally prevail and the initial reactive proliferation of the neurons gives way to a drastic loss of nerve fibres so that at the beginning of the second month most fibres have disappeared and only a few giant fibres remain between habenula and inner hair cells (Fig 4 C) (Spoendlin, 1972)

In the second month the degeneration process seems to have come to an end with the loss of 90–95% cochlear neurons (Fig 2 A) The surviving neurons are scattered throughout the cochlea and their ganglion cells are partly of the small unmyelinated type II with a lobulated and excentric nucleus and partly of type III which is a type I having lost its myelin sheath (Spoendlin, 1972, 1974) These few ganglion cells obviously provide the entire afferent nerve supply to the outer hair cells which remains entirely normal whereas the afferent innervation of the inner hair cells is almost completely lost, being associated with the 95% degenerated neurons Instead of the normal abundant radial afferent innervation of the inner hair cells a few giant fibres expanding spirally are found associated with the inner hair cells Some other fibres seem to end in the habenular openings or turn back just before the habenula (Fig 5 B) (Spoendlin, 1975)

Later the regression line becomes very flat and seems to reach an asymptote in about 2 years (Fig 2 A) Although there is a good general correlation between the number of myelinated nerve fibres in the lamina and the spiral ganglion cells, the number of nerve fibres tends to be smaller because most type II ganglion cells have unmyelinated axons The slow regression of ganglion cells between one month and about 2 years is the consequence of the long term behaviour of the surviving type III cells They are subject to constant slow regression during about 2 years, whereas the type II cells remain stable In the first year after sectioning of the cochlear nerve, the two cell types are found in about equal numbers but in the second year the type III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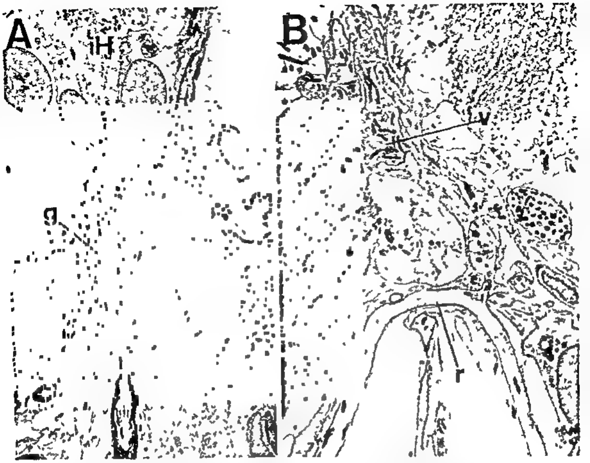

Fig 5 (A) 4 months after transection of the VIII nerve One of the surviving giant fibres (*g*) originating from a myelinated fibre penetrates through the habenula (B) 4 months after transection of the VIII nerve Some fibres end at the level of the habenula with great varicosities (*v*) filled with small vesicles whereas other fibres turn back just before the habenula (*r*)

cells diminish progressively in numbers and have almost completely disappeared by the end of the second year (Fig 2B) In many *areas especially in the upper basal and lower* second turn, exclusively type II cells are found at this late stage but even here the afferent nerve supply to the outer hair cells is entirely normal indicating its association *with the type II ganglion cells*

Fig 4 Sections through the area between habenula (*H*) and inner hair cell (*iH*) with different survival times after sectioning of the VIII nerve (A) Situation in the normal animal with the radially running afferent fibres (*a*) leading [illegible]

very large and partly spirally running fibres (*p*) Frequently enormous swellings of nerve fibres are found in the form of buds (*b*) filled with vesicles and small mitochondria The afferent fibres leading to the outer hair cells (*oh*) are unchanged at the usual place on the inner surface of the inner pillars (C) Situation 8 months after sectioning of the VIII nerve Most fibres between habenula and inner hair cells have disappeared Only a few very large giant fibres (*g*) are left in association with the inner hair cells The afferent fibres to the outer hair cells (*oh*) are found in normal numbers as basilar fibres at the bottom of the tunnel (D) Situation 20 months after transection of the VIII nerve There is an enormous number of proliferating nerve fibres below the inner hair cells (*p*) in spite of the fact that most neurons have disappeared in the spiral ganglion and osseous spiral lamina (see corresponding Figs 1C and 3B)

Degeneration starts first and proceeds most rapidly in the upper basal turn where most ganglion cells are already degenerated after 2 weeks whereas at the basal and mainly apical cochlear end even after several months survival the ganglion cell population is reduced by only about 50% (Fig 2D) In about 2 years complete degeneration has affected the entire cochlea in all turns leaving only *15–25 type II cells/100 μm and some type III* cells at the apex The remaining myelinated nerve fibres correspond in general to the type III cells Although the innervation densities vary considerably along the cochlear turns with a pronounced maximum in the basal turn and a minimum at the apex the number of surviving fibres is within the same range in all turns with a slight increase at the apex (Fig 2C) The relative percentage of the surviving neurons is therefore minimal in the basal turn and increases considerably towards the basal and especially towards the apical end of the cochlea (Fig 2D)

Comparison of the numbers of the ganglion cells in the spiral ganglion with the myelinated and unmyelinated nerve fibres in the lamina at different survival times after section of the III nerve shows a good correlation between ...linated nerve fibres and type III ganglion cells for survival times of up to about 8 months Beyond this time the number of myelinated fibres surprisingly tends to increase whereas the number of type III ganglion cells clearly decreases and the type II cells remain stable The number of myelinated axons might be more than double the number of type III ganglion cells (Fig 2B)

In those animals with long survival times usually many large myelinated fibres are seen to pass the spiral ganglion directly without an associated ganglion cell—an unknown situation in the normal animal (Fig 3) Such passing fibres are not present after short survival times and only exceptionally after selective section of the cochlear nerve They are only found when the efferents are included in the lesion of the VIII nerve This strongly suggests that they are regenerating efferent fibres which take the shortest way radially through the spiral ganglion instead of following the longer normal route of the intraganglion spiral bundle Another less probable origin of regenerating fibres could be vestibular neurons of scarpas ganglion which are less subject to retrograde degeneration and might have some regenerating capacities

Where these fibres actually grow and end is very difficult to say They certainly are neither related to the giant fibres below the inner hair cells nor to the afferent fibres associated with the outer hair cells both of which are also found after selective sectioning of the cochlear nerve in the absence of regenerating fibres They might correspond to the fibres which end with varicosities at the habenula without entering the organ of Corti or which turn back just before the habenula as if they were unable to find their way through the habenula (Fig 5B) (Spoendlin 1975)

The number of unmyelinated nerve fibres might increase considerably after sectioning of the VIII nerve with long survival times They can only be accurately assessed in electron microscopic preparations (Fig 1) In normal animals their number varies between 40 and 50/100 μm and in experimental animals it can reach more than double the normal values (Fig 2B) Their origin is multiple Mainly the larger fibres represent the axons of the type II spiral ganglion cells whereas the smaller ones probably correspond to autonomic mainly adrenergic fibres which have been demonstrated by histochemical methods (Spoendlin & Lichtensteiger 1966)

Special attention must be paid to the area of the habenula and inner hair cells where practically all normal radial fibres disappear after sectioning of the cochlear nerve and instead a few giant fibres appear which expand in a spiral direction (Fig 4C) (Spoendlin 1972 1974) They originate from myelinated fibres in the osseous spiral lamina and therefore are most likely associated with the surviving type III ganglion cells (Fig 5A)

whereas the afferent fibres to the outer hair cells seem to originate from unmyelinated fibres, most probably the axons of the type II ganglion cells After relatively short survival times from 3 to 8 months very few of such giant fibres are found Their number, however increases considerably with longer survival times over one year and in our longest survivals of 20 and 24 months these fibres have multiplied to such an enormous extent that the entire area between habenula and inner hair cells is filled and the inner hair cells are pushed aside (Fig 4D) Despite these enormous numbers of nerve fibres around the inner hair cells there are as usual only 5–10% spiral ganglion cells left These fibres must therefore be considered as an enormous proliferation of the peripheral extentions of the remaining neurons namely of the few giant fibres below the inner hair cells They cannot be regenerated efferents because they are much larger in most instances, they have afferent synaptic contacts to the inner hair cells and they develop also in animals with only the afferents cut

The vast discrepancy between the very few incoming nerve fibres to the organ of Corti through the habenula and the unusually great numbers of such fibres necessitates extensive ramifications and wide spiral extension of the incoming fibres, which in tangential sections can be directly demonstrated over distances of at least 100 μm but the limits of which have not been determined In some areas we found up to 3 000 such spiral fibres whereas in the osseous spiral lamina only about 15 myelinated nerve fibres/100 μm are found One even gets the impression that the fewer type III spiral ganglion cells and myelinated nerve fibres are present in an area the more such proliferating spiral fibres are found between habenula and inner hair cells

Even more surprising is the fact in such animals besides the normal outer spiral fibres between the Deiter cells one occasionally finds bundles of numerous spiral fibres around the base of the outer hair cells of a type similar to the fibres found below the inner hair cells This type of spiral fibre at the base of the outer hair cells is never found in normal animals They must originate from afferents of the outer hair cells since there are no other fibres leading to the outer hair cells in these animals

The long term behaviour of the cochlear nerve after its sectioning in the inner meatus reveals two so far unknown phenomena

(1) Regenerating fibres cross the spiral ganglion in the direction of the organ of Corti They probably belong to the efferents Regeneration capacities are not unexpected for the efferents since they resemble other centrifugal neurons, such as motor neurons, with well known strong regeneration power However, they seem unable to reach their final destination in the organ of Corti and probably do not have great functional significance

(2) More unexpected is the enormous proliferation of the peripheral branches of the remaining afferent neurons which seems the more pronounced the fewer neurons are left in the spiral ganglion It evokes the impression of a somewhat overshooting reaction of the remaining neurons to compensate for the loss of the great majority of cochlear neurons in order to ensure adequate nerve connections with the inner hair cells

The degeneration behaviour of the bipolar cochlear neurons does not seem to be basically different whether the central or the peripheral axon is damaged The reactions observed at the peripheral terminal branches following sectioning of the central axon might also take place in the central terminal branches at the level of the cochlear nucleus after lesions of the peripheral axons Such proliferations of terminal branches connecting the few surviving neurons to a much greater number of second order neurons might give these neurons a greater functional importance which might be useful to know in respect of the rationale of electrical stimulation of the few remaining neurons in totally deaf cochleas

RÉSUMÉ

Après section du VIII nerf cranien dans le conduit auditif interne les 95% des neurons cochleaires et toutes les fibres efferents dégenerent Après des longs temps de survie il y a des signes de regénération de certaines fibres nerveuses probablement efferentes ou vestibu laires Les rares neurons cochléaires qui restent réagissent à la lesion de leur axon central avec une proliferation énorme de leurs branches périphenques dans l organe de Corti

ZUSAMMENFASSUNG

Nach Durchtrennung des VIII Nerven im inneren Ge horgang degenerieren 95% aller Cochleansneurone und praktisch alle efferenten Fasern Nach langen Uber lebenszeiten finden sich Anhaltspunkte fur Regeneration gewisser wahrscheinlich efferenter oder vestibularer Fasern Die wenigen uberlebenden Cochleansneurone reagieren auf die Lasion ihrer zentralen Axone mit einer enormen Proliferation der peripheren Aste im Cortischen Organ

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DISCUSSION

J Tonndorf Did you—or are you going to try—to record from the nerve fibers of some of the 24 months survival animals? That would be a most important experiment

J G Hall You said that after the section of the nerve the greatest part of degeneration takes place within two months when there is a steeply falling curve flattening out between 2–4 months Now what goes on within these two first months? Or do you know when the degeneration starts?

I Friedmann This excellent study has reminded me of the great powers of regeneration of the embryonic neural elements as shown in tissue culture Could there be some such mechanism involved? The second point of interest lies in the progress proliferation of the "fibres" you have shown Have you noted any long spacing collagen that is a fairly constant ETL feature of VIII nerve tumours?

H Spoendlin (Reply) to Mr *Tonndorf* It would certainly be interesting to study the functional significance of the proliferating fibres below the inner hair cells by electrical recordings and we plan to do some recordings

To Mr *Hall* The shortest survival time we used was 2 weeks when we found an initial proliferation of the peripheral dendrites and degenerative changes in some type I spiral ganglion cells

To Mr *Friedmann* It is very well possible that the powerful capacity of long terme proliferation of the peripheral dendrite of the few surviving neurons after section of the VIII nerve is a retained embryological capacity of these neurons

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POSTURAL BEHAVIOUR AND MOTION SICKNESS

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Gifu Japan

Abstract One of the acting techniques used in Kyogen a classical Japanese stage comedy prompted two questions why is it that one may suffer from motion sickness as a car passenger but as a driver escape its effects? And how can one learn postural adjustment against motion sickness by repeatedly travelling in vehicles?

I would like to present Kyogen, the ancient and traditional stage comedy of Japan, whose style of acting prompts two questions (1) why is it that while one may suffer from motion sickness as a car passenger, it is possible to avoid it when driving? (2) How can one learn postural adjustment against motion sickness by repeatedly travelling in vehicles?

Fig 1 shows a Kyogen stage The standing actor is a boatman and the seated one is his passenger In the play, the boat appears to be moving straight from the back of the stage toward the audience The important feature in Fig 1 which I would draw attention to are the differing postures of the boatman, who is rowing and of his passenger, being ferried in the boat Now let me show you by figures The standing boatman stretches out his arms to push the oar, bending forward, his posture inclines to your left In contrast, the seated passenger inclines to your right, i e in the opposite direction Thus the inclinations of the two postures are mutually counterposed

As the rowing action continues, the postural inclinations of the two actors interchange as shown in Fig 2 The boatman who is rowing the boat bends backward, i e the posture inclines to your right while the posture of his passenger inclines to your left This alternating of posture continues rhythmically to simulate the action of rowing When, on the stage the boatman and his passenger move rhythmically in opposite directions, the audience feels just as if they are watching a boatman and a passenger in a real boat, hurrying on its way through the waves

Fig 1 The boa[illegible]

Fig 2 As the rowing action continues the direction of each postural inclination changes to the opposite side The head and trunk of the boatman who is pulling the oar bend backward i e the posture inclines toward your right while the passenger inclines toward your left Thus the two postures move into opposite directions

Similar contrasts in bodily postures, such as at illustrated for boatman and passenger, can observed in our daily life For example, as hown in Fig 3, the head and trunk of a bus driver incline toward the right when he turns the wheel to the right in order to turn the bus to the right, while the head and trunk of a passenger in the same bus incline toward the left Thus the postures of the two individuals incline in opposite directions From the viewpoint of centrifugal force, it is interesting to note that the driver leans across the midline, against the centrifugal force, taking a centripetal stance The passenger, however, leans across the midline at an inclination counter to the turning of the bus and displays a centrifugal posture It is very interesting to note that these contrasting postures are not illusory stage artefacts but real physiological phenomena caused by the movement of a bus which swings to the right

I would like to emphasize that such mutually antagonistic patterns of posture as seen with driver and passenger in a moving bus as well as with boatman and passenger in a boat on a symbolic stage of ■ Kyogen is closely related to the cause of motion sickness

Guttich (1940) observed that the behaviour of the human head and eyes differs in active vs passive rotation In an active rotation, the eyes and head move in the direction of rotation, while in a passive rotation, they move counter to the direction of rotation—of a chair, for instance In this way, each position assumes an antagonistic and symmetric posture over the midline of the body This antagonism is observed not only during rotatory movement around one's own axis but also during other linear and circular movements and it constitutes a general rule of posture, differentiating active and passive movements of human beings

Fig 3 The head and trunk of the bus driver incline toward your right when he turns the steering wheel in a counter clockwise direction to make a right turn while the head and trunk of the passenger in the same bus incline toward your left Thus the two postures move in mutually opposite directions

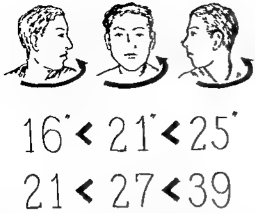

Fig. 4. With head turned and fixed toward the direction of chair rotation the mean value of duration in seconds and the number of beats of postrotatory nystagmus decreased markedly compared with the case in which the head was fixed in straight head position. By contrast with head rotated and fixed opposite the direction of chair rotation it increased markedly. Arrow: direction of chair rotation.

Now, returning to the question posed above: why a person when driving a car does not suffer from motion sickness while he may do so when he is a passenger in a car. To answer this question, the following experiments were performed (Fig. 4).

Postrotatory nystagmus after 10 rotations in 20 seconds was adopted as an indicater of motion sickness. Seventy healthy adults, male and female, were rotated 10 times in 20 seconds on a rotating chair in a counter-clockwise direction. During the rotation the eyes were closed and the head was fixed in three different positions as follows: normal, i.e. 30° prone position from the German hosizontal line; extreme left, i.e. in the direction of chair rotation; extreme right, i.e. counter to the direction of chair rotation.

The results are as follows. In the case of normal head position the numerical mean value of postrotatory nystagmus for 70 subjects was 27 beats in 21 seconds. With the head positioned extreme left it was 21 beats in 16 seconds and with the head positioned extreme right it was 39 beats in 25 seconds. To summarize: by fixing the head toward the direction of chair rotation the mean value of duration and beats of postrotatory nystagmus decreased markedly compared with the effect of fixing the head in normal head position. In contrast, by fixing the head against the direction of chair rotation, nystagmus increased appreciably.

Now, returning to Fig. 3—driver and passenger in a bus—I would like to answer the first question. The posture of a driver may be said to correspond with the head position fixed toward the direction of chair rotation, i.e. position of the head resistant to postrotatory nystagmus, in other words, a posture in which one does not easily develop motion sickness. On the other hand, the posture of a passenger may be said to correspond to the head position fixed counter to the direction of chair rotation and thus vulnerable to postrotatory nystagmus—that is, a posture in which one easily develops motion sickness. Thus the marked decrease and increase in postrotatory nystagmus observed in the above experiments clearly explains why the driver of a car hardly

Fig. 5. Posture of an experienced bus-conductress while the bus is making a right turn. Even when moved passively in the bus, her posture inclines toward the right, i.e. in the same direction as that of the driver and opposite to that of the passenger shown in Fig. 3.

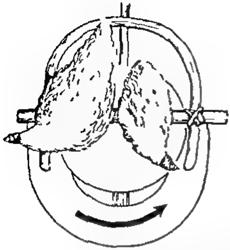

Fig 6 Trained (*right*) and untrained (*left*) chickens 10 rotations after the beginning of rotation The head of the trained chicken turns in the same direction as chair rotation through the kinetic labyrinthine reflex while the head of the untrained bird still deviates in the direction opposite to rotation by the static labyrinthine reflex i e the *Kopfremanenz* of Ewald

ever suffers from motion sickness while a passenger may easily do so

Now, let me answer the second question—on the problem of trained postural adjustment against motion sickness, or habituation It is well known that sailors and crews o have accumulated many years of experi- ce in traveling on vessels do not suffer from motion sickness These people have developed special postural habits, an example of which is illustrated in Fig 5 I observed the posture of a trained busconductress who had been working on a bus for 5 years and made photographs of her back without her knowledge, while the bus was turning When the bus swung to the right, she assumed a posture with head and trunk inclined toward the right namely in exactly the same direction as the inclination of the driver and opposite to that of the passenger in Fig 3 It is very interesting to note that the passenger and the bus-conductress, while sitting in the bus adopted mutually quite opposite postures and inclined in opposite directions to each other The bus conductress adopted a posture similar to that of the driver whose posture was shown to be resistant to motion sickness Thus a trained posture or habituation is objectively demonstrated in the bus conductress who assumed an actively inclined posture toward the centre of a curve, i e a centripetal posture

The centripetal posture was reported by the author under the title "Static and kinetic labyrinthine reflex" To summarize the principal points shown in Fig 6, blindfolded chickens were rotated 100 times in 200 seconds in both directions every day for 2 weeks It was observed after repeated rotations, that during rotation the birds turned their heads in the direction of rotation This phenomenon was never found in birds prior to repeated exposure to rotation The labyrinthine reflex which caused this behaviour has been named the "Kinetic labyrinthint reflex" In contrast to this terminology, the deviation of the head against the direction of rotation has been called the "Static labyrinthine reflex" or "*Kopfremanenz*" by Ewald to maintain the original position of the head The birds showed functional progress in equilibrating function through repeated exposures to rotation, which were therefore termed "Training Thus a trained postural behaviour during rotation was demonstrated objectively in the establishment of a kinetic labyrinthine reflex which is again here clearly observed in the posture of the trained bus conductress

RÉSUMÉ

Une étude sur le style intéressant du jeu théatral du Kyogen la comédie classique du Japon a donné les réponses aux questions suivantes 1) Pourquoi n a t on pas le mal de la route quand on conduit le véhicule tandis que l on peut en souffrir quand on est passager? 2) Comment apprend on à adopter une posture convenable pour éviter le mal de la route à travers plusieurs voyages en véhicule?

ZUSAMMENFASSUNG

Ein interessanter Stil des Spiels auf der Buhne von Kyogen eine klassische Komodie des Japans wurde gezeigt um die folgenden zwei Fragen aufzuklaren 1) Warum bekommt man nicht den Schwindel wenn er selbst ein Auto fährt wahrend er davon befallen werden kann wenn er ein Fahrgast ist? 2) Wie lernt man die *Anpassungsfähigkeit der Korperhaltung vom Schwindel* befreit zu werden durch die wiederholten Fahrten?

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DISCUSSION

L B W Jongkees I hope that your observations will be of help for those who suffer from seasickness though I do not yet see in what manner

J Tonndorf I think one cannot argue with your beautiful observations but I like to differ with you as regards the interpretation I consider the whole phenomenon that you demonstrated as a stato-vestibular one not a semicircular-canal effect I say that mainly on account of the large turning radius which permits of only minimal semicircular stimulation I think what the trained person does is to adjust his head position to the horizon while the untrained person goes with the centrifugal force W Johnson (Toronto) has shown that in his swing experiments As long as the subject is able to keep his head steady he is alright When he lets his head go he becomes motionsick

M Arslan The important factor of the motion sickness is certainly the Coriolis Cross Coupling Effect I have performed some experiments on the effects upon the neurovegetative innervation of the cardiovascular system The results showed an excitation of the reticular efferent pathways either sympathetic or parasympathetic A mathematical model of the forces provoked in the vestibular endolymph (realized by dr Blezzo physician) is presented

W Oosterveld You suggest that car sickness is due to the very small angular accelerations applied to passengers when a car makes a turn However people become car sick even if a car does not make turns at all It is known that vibration plays an important role in the origin of car sickness Perhaps the attention paid by the passengers to the movements of a car increases the threshold to become carsick

T Fukuda (Reply) to Mr Oosterveld after having thanked Mr Jongkees Mr Tonndorf and Mr Arslan for their kind remarks Of course motionsickness is not caused only by rotatory movements I showed the results of postrotatory nystagmus because it gives a numerical data of an artificial labyrinthine ataxia i e a motion sickness which shows the grade with the number of beats and its duration

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BACTEREMIA AFTER TONSILLECTOMY AND ADENECTOMY

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Abstract 40% of negative hemocultures become positive immediately after tonsillectomies and/or adenectomies This bacteremia is asymptomatic and remains no longer than one hour

Some authors, after Seifert (1925), have reported bacteremia following gynecologic or urologic surgery and after dental extractions (Lehman, 1926, Scott, 1929, Barrington & Wright, 1930) Other authors tried to demonstrate this type of bacteremia after tonsillectomy, with contradictory results (Schwartz & Frisch, 1929, Bartlett & Pratt, 1931, Fischer Gottdenker, 1936)

We made a similar investigation in a series of 100 tonsillectomies/adenectomies, or both operations performed together, on children and adults, under general or local anesthesia *The hemocultures of these patients were regu*larly negative before the operation No use of antibiotics As soon as possible, within 5 minutes after the operation, the first 10–20 cc of blood was drawn from an arm vein and injected into 400 cc of peptone broth which is known to allow, thanks to the layer of red cells, the growth of both aerobic and anaerobic microbes We checked the cultures every 3 days

Smears of the removed tonsils and adenoids and the organs themselves were also cultured

From the 100 hemocultures checked we obtained 40 positive results, detailed as follows

85 tonsillectomies and adenectomies performed together	35+
10 tonsillectomies	3+
5 adenectomies	2+

The 40 positive hemocultures showed 1 type of microbe in 29 cases, 2 types in 9 cases, 3 types in 1 case and 4 types in 1 case The most frequently detected microbes were *Gaffkya tetragena* (16 cases) and *Streptococcus pneumoniae* (11 cases)

None of the 40 patients having a positive hemoculture developed clinical symptoms even when the microbe was *Streptococcus hemolyticus* (4 cases) or *Streptococcus viridans* (3 cases) This lack of clinical symptoms marks the difference between bacteremia and septicemia

Table *Species of microbes and frequency of detection in the 40 positive hemocultures*

Gaffkya tetragena	in 16 cases
Streptococcus pheumoniae	in 11 cases
Haemophilus influenzae	in 4 cases
Corynebacterium pseudo-diphtheriticum	in 4 cases
Spreptococcus haemolyticus	in 4 cases
Streptococcus viridans	in 3 cases
Neisseria catarrhalis	in 3 cases
Staphylococcus aureus	in 2 cases
Streptococcus anhaemolyticus	in 1 case
Neisseria flavescens	in 1 case
Escherichia coli	in 1 case
Mycotorula albicans	in 1 case
Pseudomonas aeruginosa	in 1 case
Bacillus mesentericus	in 1 case
Streptococcus anaerobius	in 1 case

In all the cases of positive hemoculture, we found at least one of the microbes present in the smear cultures and in the cultures of the organs themselves We never found repeated hemocultures remaining positive longer than 1½ hours, the average being 1 hour

The mechanism of the defensive reaction could be explained by the following experiment When guinea pigs are injected with a microbial suspension, the latter becomes rapidly agglutinated to the plaquettes and then blockaded in the deep capillaries where they are phagocytosed Furthermore, this mechanism is made easier by encapsulated microbes which are the most frequently detected in the positive hemocultures

RÉSUMÉ

40% des hémocultures négatives avant une amygdalectomie et/ou une adénoidectomie deviennent positives dans les minutes qui suivent l opération Cette bactériémie est asymptomatique et ne persiste pas plus d'une heure en moyenne

ZUSAMMENFASSUNG

40% von negativen Hemokulturen werden positiv unmittelbar nach Mandeloperationen und/oder Adenoidektomien Diese Bakteriemie ist asymtomatisch und halt nur eine Stunde an

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DISCUSSION

L B W Jongkees Is it possible that the fact that the number of patients who show positive bacterial findings after tonsillectomy has gone down in the last forty years (compare Hulk about 1930 70% of bacteriemia) is due to the increasing influence of viruses in chronic tonsillitis?

A Montandon Van Eyck a t il pu detecter apres coup de cette maniere un porteur de germes latent de streptocoque B hemolytique? Et y a t il une correlation entre la bacteriemie postoperatoire et le tauxanquin des antistreptolysines?

T Leegaard During the last five years before my retirement we treated all patients with peritonsillar absess with instant tonsillectomy and for the first two years we made routinely a blood culture In only one case out of a considerable number we had growth of bacteria not without any clinical symptoms

M Van Eyck (Reply) to Mr *Jongkees* I know that work by *Hulk* done in 1937 The results were more often positive than ours The explanation about viral infection could be the good one

To Mr *Montandon* Les examens de sang preoperatoires comportaient les antistreptolysines Il n y avait pas de correlation entre les resultats des frottis d organes et ces antistreptolysines ni avec les resultats des hemocultures Il n y avait pas necessairement une bacteriemie postoperatoire dans les cas d elevation des antistreptolysines dans le sang ni le contraire

To Mr *Leegaard* As I heard blood cultures followed tonsillectomies in acute phase of infection and so the chances to get a positive hemoculture could be greater than in our cases operated "a froid" It seems not to be the fact Perhaps is it a question of bacteriological technic which is somewhat delicate

Acta Otolaryngol 81 244–248, 1976

TECHNICAL VARIATIONS IN CLINICAL OSTIAL PATENCY TESTS

A E Kortekangas and T Rantanen

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Abstract The usefulness of nasopharyngeal–antral pressure difference recording as a part of clinical ostial patency testing is demonstrated by statistical calculations from 507 patency tests This method offers a quick confirmation of the comparison of pressure variation measured by anterior rhinomanometry and by antral pressure recordings which are the main elements of a consecutive patency test In addition the nasopharyngeal–antral recording has many of the advantages of a simultaneous patency test When this difference recording is applied the nasal reference pressure recording can also be omitted without any loss of information sought by the patency test

Maxillary sinus ostia were found to be perma[illegible] openings in cadavers in experiments [illegible] by Braune & Clasen (1877) Re[illegible] of clinical observations on ostial patency appeared a good deal later on (Doderlein 1932 Proetz 1932 Schmucker 1932 Kerekes 1934 Muller 1938)

DISCUSSION OF METHODS

In the more recent studies two principally different methods of performing the patency test have been used Drettner (1965) used two manometers one connected to the sinus lumen and the other recording from the nasal cavity as near the nasal opening of the maxillary ostial canal as possible This is the physically accurate *simultaneous patency test* (Fig 1)

Recording from the maxillary sinus presupposes in any case a sinus puncture which is often connected with some inconvenience to the patient A proper recording from the nasal cavity at the nasal opening of the maxillary sinus ostium is also inconvenient to the patient Many patients find it even more unpleasant than the maxillary sinus puncture This inconvenience is the apparent reason for proposals to use anterior rhinomanometry instead of intranasal recording, as the nasal reference recording (Cottle 1968 Rantanen & Kortekangas 1971) Such a procedure however, does not allow a simultaneous recording The nasal reference recording and recording from the maxillary sinus must be made one after another (Fig 2) Accordingly this type of patency test is called a *consecutive patency test*

In addition to the differences due to possible variation in the amplitude of the nasal pressure fluctuation during the recording time the pressure change that originates in the posterior part of the nasal cavity causes a difference between the reference and the antral recording in the consecutive test It has been shown however that the contribution of the posterior nasal cavity is only very small compared with the pressure change created in the anterior part of the nasal cavity (Fischer 1969) This finding is the main argument in favour of the physically less accurate but clinically much more convenient consecutive patency test

To combine the clinical applicability of the consecutive test and some of the exactness of the simultaneous patency test a nasopharyn

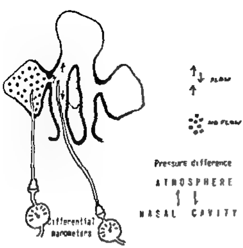

Fig 1 Simultaneous patency test

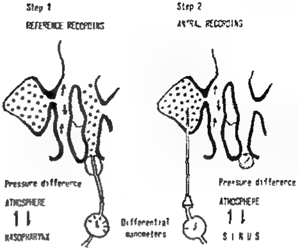

Fig 2 Consecutive patency test

geal-antral difference recording was proposed (Kortekangas, 1970) During this recording, both sides of a manometer (pressure transducer) are utilised. One side is connected to the sinus and the other side to the nasopharynx as in anterior rhinomanometry (Fig 3) If the respiratory pressure fluctuation is equal inside the sinus and nasopharynx, there is no fluctuation during the nasopharyngeal-antral pressure difference recording During this recording the pressure change created in the posterior nasal cavity is likewise ignored as the antral pressure must equal the pressure at the nasal orifice of the maxillary sinus in the nasal cavity, not that in the nasopharynx

Recordings characteristic of each type of ostial patency are given in Fig 4 The most frequently measured parameter is the inspiratory peak value The corresponding expiratory value would give almost identical results For a patent ostium a 25% difference is allowed between the reference recording and the antral recording (Rantanen, 1974) In some cases, especially when the peak pressures are low, it may be difficult to decide the type of patency by comparing reference and antral recordings When the nasopharyngeal–antral difference recording is included in the test, a quick confirmation is gained This is very valuable when the test is used to evaluate the ostial patency in practical clinical work When the nasopharyngeal–antral pressure difference is applied, the reference recording can also be omitted and the patency determined directly from antral and nasopharyngeal–antral recording by glancing at the graphs or by watching the movements of the manometer needle

To further study the value of nasopharyngeal–antral difference recording, comparisons were made between reference (nasopharyngeal-ambient) recording, antral (antral-ambient) recording and nasopharyngeal–antral

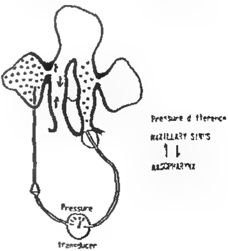

Fig 3 Nasopharyngeal–antral pressure difference recording

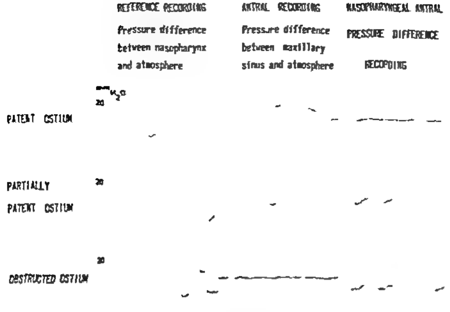

Fig 4 Maxillary sinus ostium patency test Pressure recordings in different types of patency

difference recording The inspiratory mean peak pressure fall of five consecutive breaths was used as the *parameter*

If the nose, the maxillary sinus and its ostia were permanently rigid structures the sum of antral pressure and nasopharyngeal–antral difference should equal the reference pressure in the nasal cavity This too is the only meas-
...ment in our testing arrangement that is
...e for simple comparison The reference pressure was recorded only at the beginning of each test The antral pressure and nasopharyngeal–antral pressure difference were recorded three times in conjunction with the sinus puncture, natively, after cleansing of the trocar by suction, and after irrigation of the sinus As several procedures were performed during each phase, the recording may well be different in some details from the previous one, especially in relation to the mobile external pyramid of the nose In addition, changes affecting the pressures inside the nose must also be expected

RESULTS

Table I gives a comparison in relation to the clinical patency of the ostia This was determined by comparing the antral and refer

Table I *Comparison of mean inspiratory nasal reference pressure peak fall with the sum of mean inspiratory antral pressure peak fall and nasopharyngeal–antral peak difference (diff) in the three types of clinical patency of maxillary sinus ostium*

	Statistically equal *n* (%)	Reference greater than *antral+diff* *n* (%)	Reference smaller than *antral+diff* *n* (%)	Total no of estimates
Patent ostium	102 (62 5)	4 (2 5)	57 (35 0)	163
Partially patent ostium	60 (50 0)	41 (39 2)	19 (15 8)	120
Obstructed ostium	145 (64 7)	20 (8 9)	59 (26 4)	224
Totals	307 (60 6)	65 (12 8)	135 (26 6)	507

Table II *Comparison of the mean inspiratory peak pressure fall during reference (nasopharyngeal ambient) recording to the sum of antral (antral ambient) and nasopharyngeal–antral difference (diff) recording*

Percentage distribution in different statistically determined types of ostial patency

	Natively	Post suction ally	After irriga tion
Reference = antral (statistically patent ostia)			
No of observations	41	46	46
Reference = antral + diff	83%	78%	85%
Reference > antral + diff	0	0	0
Reference < antral + diff	17%	22%	15%
Reference < antral (classified as patent ostia)			
No of observations	10	13	9
Reference = antral + diff	0	0	0
Reference > antral + diff	0	0	0
Reference < antral + diff	100%	100%	100%
Reference > antral (statistically partially patent ostia)			
No of observations	39	42	37
Reference = antral + diff	41%	45%	49%
Reference > antral + diff	49%	33%	32%
Reference < antral + diff	10%	22%	19%
Reference > antral (= 0) (statistically obstructed ostia)			
No of observations	79	68	77
Reference = antral + diff	73%	60%	60%
Reference > antral + diff	9%	9%	9%
Reference < antral + diff	18%	31%	31%

ence recording, allowing a 25% difference in the mean peak value The distributions were similar in the different phases of the examination and therefore only summation frequences are given in Table I Table II gives a similar comparison of statistically calculated ostial patency This calculation is made between reference pressure and antral pressure similarly by determining the means of five consecutive inspiratory peak values

The agreement in these results must be considered very good as so many interfering factors are present during the tests A good example of such factors is the number of observations in which antral pressure was greater than the reference pressure This does not affect the present problem, the reliability of nasopharyngeal–antral pressure difference recording but is a sign of inevitable variations in investigations like this

We consider our findings to confirm that nasopharyngeal–antral difference recording as a quick and physically fairly accurate method is a valuable contribution to the clinical testing of ostial patency

RÉSUMÉ

L obstruction de l orifice est souvent considérée comme le point le plus critique du « cercle vicieux » qui résultera dans une sinusit manifeste La méthode la plus sensitive pour étudier la fonction de l'orifice est de verifier la perméabilité de l orifice du sinus maxillaire Ce rapport est une étude comparative des méthodes successives et simultanées

ZUSAMMENFASSUNG

Die Verwendbarkeit der nasopharyngeal antralen Druck differenzmessung als eine Erganzung zur klinischen Durchgangigkeitsbestimmung ist bei 507 solchen Bestimmungen gepruft worden Durch dieses Verfahren kann das Resultat der Vergleichung von hintereinander bestimmten antralen und nasopharyngealen Druckvariationen schnell uberpruft werden Die nasopharyngeal–antrale Druck differenzmessung bietet manche Vorteile einer Simultanmessung und wenn eine solche Messung in die klinische Durchgangigkeitsbestimmung einverleibt ist kann die nasopharyngeale Referenzmessung sogar vernachlassigt werden

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DISCUSSION

J Tonndorf I believe you said that, theoretically, the pressures on both sides, in the nose and in the sinus should be equal I don t think this is necessarily correct 1) The pressure in the sinus is measured at the end of a resistor, the ostium Therefore, there is an IR drop, i e , the pressure within the sinus should always be the smaller of the two 2) On account of two reactive factors (a) the mass of the air moved and (b) the elasticity of the nasal walls, there should be a phase difference which will also keep the two pressures from being equal at the same instant

B Drettner The patency test is a valuable method for qualitative studies of the patency of the maxillary ostium When using the simultaneous patency test we also have the possibility to measure the resistance of the ostium in these cases where the ostium works as valve Have you also this possibility when using a consecutive procedure? Real quantitative methods have a definite value as a complement to the patency test For that purpose we have in Uppsala elaborated two different quantitative tests for the maxillary ostium 1) For measurements of the resistance of obstructed ostia, 2) for measurements of the functional size of patent ostia The latter method gives for example the opportunity to reveal if a patent ostium is narrow or not Since you have reported operations with the purpose to enlarge the maxillary ostium and since you also have reported cases with chronic sinusitis who have had patent ostia I would like to ask you if these cases had narrow but still patent ostia?

A E Kortekangas (Reply) to Mr *Tonndorf* I completely agree that there is a physical inaccuracy in our testing procedure, which is ment purely for clinical purposes The time difference is very small if the ostium has a reasonable size, our clinical and model observations agree with the earlier physical measurements This time difference does not interfere with the result in this consecutive test as the parameter was consecutive peak values In the nasopharyngeal-antral pressure difference recording this time difference is hardly seen which proves its insignificant effect I think that the physical inaccuracy is well covered by the normal variation inevitably bound to clinical observations

To Mr *Drettner* I would like to recommend that the term resistance is reserved only for those tests in which saline or gas is made to flow through the ostium and the overpressure necessary to initiate this flow is used as a measure of the resistance So the patency test does not measure this parameter, nor can it be estimated from the graphs when forced respiratory efforts are used to change the patency in our testing procedure To the question on valve phenomenon I would like to inform of our clinical observations based on model experiments by my colleague Rantanen According to those the valve phenomenon can be caused by secretion and also probably is in most cases due to that A thin-bubble moving in the ostial canal can have such an effect

Our testing procedure is not suitable for measuring the size of the ostium It is only a qualitative test

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SKULLS WITH FACIAL CLEFTS

Experimental Surgery on the Facial Skeleton

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Abstract Skulls of adult unoperated patients with facial clefts show additional anomalies specific for the type of cleft In an experimental study we demonstrated that similar anomalies develop in rabbits with clefts that are artificially made in the fourth week after birth Some surgical methods (Veau primary osteoplasty) for correction of facial clefts are compared with regard to their effect upon later growth of the skull of young rabbits The importance of experimental surgery preceding the introduction of new surgical techniques on the growing skull and its facial skeleton in particular is stressed

As nearly all children with facial clefts receive surgical and orthodontic treatment there is very little information on the spontaneous development of untreated cleft affected skulls In the anatomical museums of Amsterdam and Utrecht 20 skulls with facial clefts were found dating from before the introduction of treatment of clefts in the Low Countries (van Limborgh 1964)

The adult skulls showed patterns of anomalies characteristic for the type of cleft (Table I) For instance in adult skulls with unilateral cleft lip alveolus and palate the premaxilla and maxilla on the non cleft side are displaced from the midline The maxilla on the cleft side shows a medial collapse and a retroposition also the adjoining pterygoid process is displaced posteriorly (Fig 1)

In neonatal skulls these specific patterns of anomalies could not be demonstrated so we got the impression that they develop in the period between birth and adult age

The question arises as to whether this late abnormal development is primary—an intrinsic part of a ' cleft syndrome , or secondary—a reaction of the growing skull to the presence of the cleft The answer to this question, being of importance for the evaluation of skull growth after surgical correction of clefts was the object of a first series of experiments

Growth of skull in rabbits with surgically produced facial clefts

In 4 weeks-old healthy rabbits, three types of facial cleft were produced by surgery (Fig 2) Twenty weeks later the adult animals were sacrificed Their skulls were prepared and studied morphologically and geometrically in

Table I *Anomalies in skulls of human adults with untreated facial clefts unilateral cleft alveolus (I CA) unilateral cleft alveolus and palate (I CAP) and solitary cleft palate (CP) modified from van Limborgh (1964)*

	I-CA	I-CAP	CP
Premaxilla			
rotation	+	+	−
deviation to non-cleft side	+	+	−
Maxilla non-cleft side			
deviation from midline		+	
Maxilla cleft side			
retroposition		+	
Decrease in palatal width	−	+	+
Pterygoid process cleft side			
retroposition	−	+	−

Fig 1 Part of the skull of a human adult with an untreated unilateral cleft alveolus and palate (Museum Vrolik Dept of Anatomy and Embryology Univ of Amsterdam) Deviation from the midline of the premaxilla and maxilla on the non cleft side Medial collapse and retroposition of the maxilla and pterygoid process on the cleft side

comparison with a control group of 24 weeks-old non operated rabbits

The geometrical study included two types of measurements 1) In order to describe the position of the maxillary complex and the premaxilla in quantative terms the points *P Q S T* and *U* were marked on lateral standardized photographs and defined by construction of a rectangular coordinate system based on the occiput (Fig 3) 2) In order to determine the palatal width the distances between the molars in the two alveolar processes were measured directly on the skull through a microscope

As the sizes of the skulls differ considerably, a normalizing procedure was used which allowed us to compare all the experimental skulls with the control group The findings have been evaluated for statistical significance by applying a multi variate analysis according to Hotelling (Verwoerd-Verhoef, 1974, Urbanus, 1974)

The most important observations in the adult animals with a cleft lip and alveolus (Fig 4*b*) were the following A rotation of the premaxilla to the cleft affected side, a deviation of the anterior part of the skull (premaxilla and

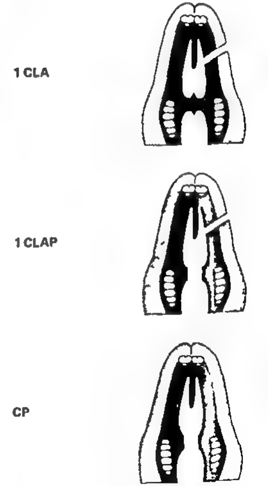

Fig 2 Schematic drawing of the caudal side of the upper jaw showing 3 different facial clefts which were surgically produced *1 CLA*=unilateral cleft lip and alveolus *1-CLAP*=unilateral cleft lip alveolus and palate *CP*=cleft palate The premaxillo maxillary suture and/or median palatal suture are excised

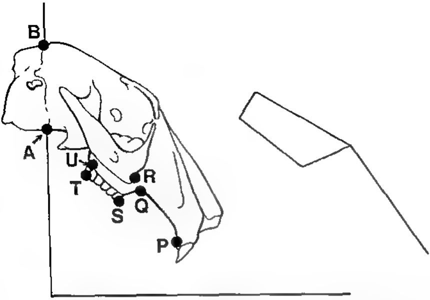

Fig 3 Lateral side of the skull of a rabbit The points *P U* were defined by construction of a rectangular coordinate system The ordinate is formed by the reference line through the most caudal point of the spheno-occipital synchondrosis (*A*) and the most cranial point in the lambdoid suture (*B*) The abscissa is a perpendicular line in *A* (not shown in the scheme)

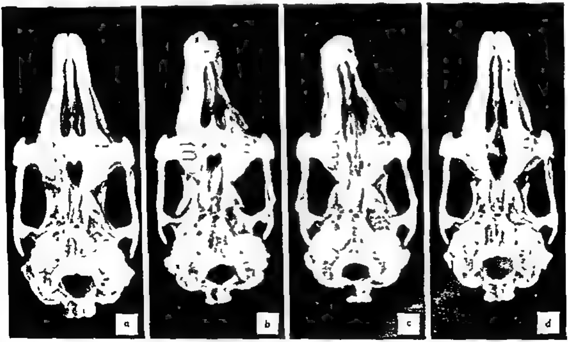

Fig 4 Caudal sides of the skulls of 24-weeks-old rabbits (*a*) Non-operated control (*b*) Unilateral cleft lip and alveolus rotation and deviation from the midline of the premaxilla retroposition of the maxillary complex on the cleft side (*c*) Unilateral cleft lip alveolus and palate rotation and deviation from the midline of the premaxilla deviation of the maxilla on the cleft side retroposition and medial collapse of the maxilla zygoma and pterygoid process on the cleft side Compare with Fig 1

Fig 1 Part of the skull of a human adult with an untreated unilateral cleft alveolus and palate (Museum Vrolik Dept of Anatomy and Embryology Univ of Amsterdam) Deviation from the midline of the premaxilla and maxilla on the non-cleft side Medial collapse and retroposition of the maxilla and pterygoid process on the cleft side

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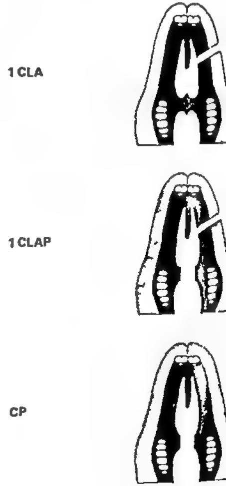

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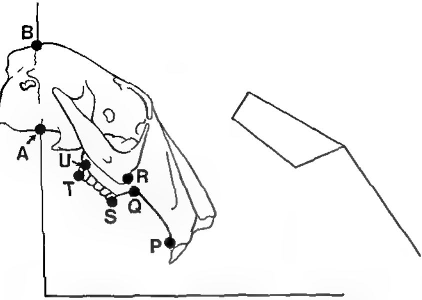

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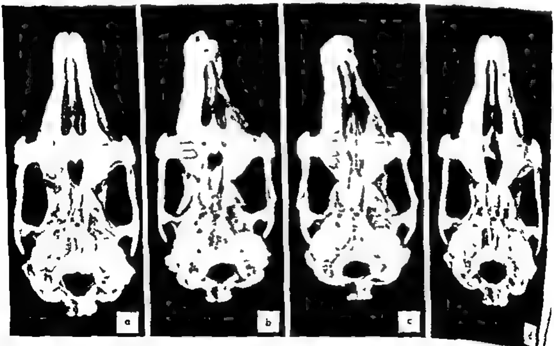

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planing

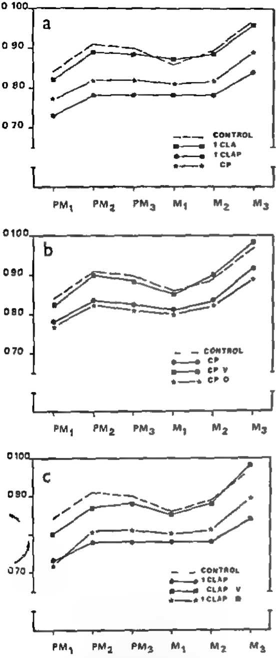

Fig 5 Graphic representation of the mean normalized palatal width (*ordinate*) between the premolars (PM1–PM3) and molars (M1–M3) (*abscissa*) in 24 weeks

Closure of the solitary cleft palate according to Veau (*CP V*) prevents a decrease in palatal width in comparison with the control After the primary osteoplasty (*CP O*) the palatal width is reduced to the same extent as in animals with a non-corrected cleft palate (*CP*) (*c*) Closure of a unilateral cleft lip alveolus and palate according to Veau (*I CLAP V*) prevents a decrease in palatal width in contrast to closure by primary osteoplasty (*I-CLAP-O*)

nasal bone) to the non cleft side, and a slight posterior displacement (i e retroposition) of the maxillary complex on the cleft side The combination of cleft lip and alveolus with a cleft palate (Fig 4*c*) also resulted in a rotation of the premaxilla The nose, including the ventral part of the maxilla, is deflected to the non operated side The retroposition of the maxillary complex and zygomatic bone on the cleft side is more pronounced than in skulls with clefts of lip and jaw only In addition the palatal width was diminished (i e medial collapse) Skulls with a solitary median cleft of the palate (Fig 4*d*) showed no signs of asymmetry but were characterized by a significant degree of medial collapse (Fig 5*a*)

From this series of experiments we concluded that the presence of facial clefts causes an abnormal development of the skull in the rabbit which is highly constant and specific for the type of cleft Moreover, the observed anomalies are very similar to those seen in human skulls with corresponding untreated clefts Consequently we may consider the anomalies in skulls of untreated adult patients to be secondary to the presence of the cleft The experimental results are contradictory to the assumption that the deformities are an intrinsic part of the cleft syndrome

The next question was whether surgical closure of the cleft could prevent this secondary abnormal growth of the cleft affected skulls and further, whether in this respect there is a difference between the clinically used methods of Veau and Schuchardt (primary osteoplasty) This was investigated by applying both methods, slightly modified, to young rabbits with facial clefts

Growth of rabbit skull with surgically closed facial cleft

In 4 weeks-old rabbits three types of facial clefts unilateral cleft lip and alveolus unilateral cleft lip, alveolus and palate and *solitary cleft palate* were surgically produced and closed in the same session following the principles of the methods of Veau and Schu

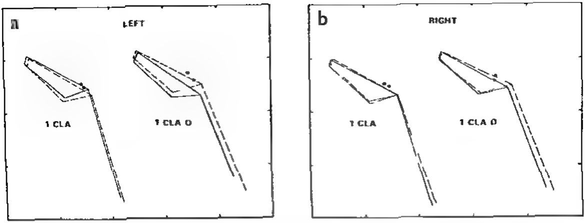

Fig 6 Graphic representation of the mean normalized coordinates of the points *P U* in skulls of 24 weeks-old rabbits with a unilateral cleft lip and alveolus (*I-CLA*) and with the same facial cleft closed by primary osteoplasty (*I-CLA O*) (—) compared with a control group ( ) The most rostral point of the zygoma in *I-CLA* and *I-CLA O* is indicated by • in the control by ★ (*a*) Left (operated) side retroposition of the molar complex and zygoma in *I-CLA* in contrast to an anteroposition in *I-CLA-O* (*b*) Right (non-operated) side nearly identical positions of the molar complex in *I CLA I-CLA-O* and control

chardt According to Veau the cleft was closed in 2 layers a nasal and an oral mucoperiost layer At the primary osteoplasty, autogenous bone was implanted between the two layers as well

At the adult stage (24 weeks) the skulls were studied morphologically and geometrically in comparison with skulls of rabbits with un treated clefts, and with a control group

In the cleft palate, solitary or in combination with a unilateral cleft lip and alveolus, only Veau's procedure could prevent the development of a medial collapse (Fig 5*b*, 5*c*) In the unilateral cleft lip and alveolus and the uni lateral cleft lip, alveolus and palate, the out-growth of the upper jaw was much better after the Veau procedure than after an osteoplasty If a unilateral cleft lip and alveolus is closed according to Veau, there is a slight deviation of the premaxilla to the cleft side, and an anteroposition of the maxillary complex on the cleft side The primary osteoplasty results in a prominent deviation of the nose to the operated side an anteroposition of the maxil lary complex of the cleft side, and a shortening of the upper jaw (Fig 6)

When both methods are applied to the uni

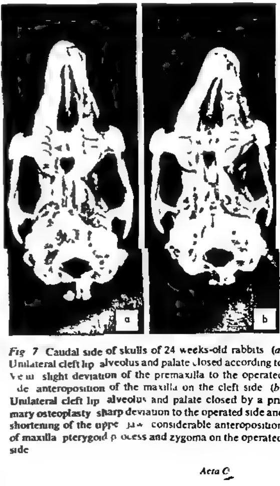

Fig 7 Caudal side of skulls of 24 weeks-old rabbits (*a*) Unilateral cleft lip alveolus and palate closed according to Veau slight deviation of the premaxilla to the operated side anteroposition of the maxilla on the cleft side (*b*) Unilateral cleft lip alveolus and palate closed by a primary osteoplasty sharp deviation to the operated side and shortening of the upper jaw considerable anteroposition of maxilla pterygoid process and zygoma on the operated side

Fig 8 Lateral view of the skull of an adult rabbit with a cleft lip alveolus and palate, closed by primary osteoplasty The shortening of the upper jaw results in a frontal malocclusion

lateral cleft lip, alveolus and palate, essentially the same differences are observed but to a much greater extent (Fig 7) The shortening of the upper jaw results in a considerable frontal malocclusion (Fig 8) The deviation of the nose to the operated side is not restricted to its frontal part (rostral to the hard palate) but includes the entire maxilla

In case of a solitary cleft palate, only Veau's method gave satisfactory results, being followed by a 'normalized' development of the ll Both methods tested for closure of unilateral clefts introduced other characteristic divergences in skull development than observed in animals with untreated clefts The results of the Veau's procedure are superior to those of the primary osteoplasty as regards the subsequent development of the skull The severe disturbances of skull development after the latter were unexpected This surgical technique was introduced in 1964 as a one stage operation to restore the upper jaw to normal but the conceptual base of the primary osteoplasty has proved false

In the jaw with a cleft a suture is lacking A suture ensures continuity and permits lengthening at the same time By osteoplasty the continuity is restored with autogenous bone, but since there is no suture lengthening is made impossible This causes a sharp deviation and even a shortening of the outgrowing upper jaw

The observations made in experimental animals could have predicted the only recently reported disappointing growth of the skull in children who are treated with a primary osteoplasty (Robertson & Jolleys, 1971 Friede & Johanson, 1973, Schmid et al , 1973) This stresses the importance of experimental surgery prior to the introduction of new surgical techniques on the growing skull and the facial skeleton, especially in children

In this paper the results of many experiments are brought together in order to focus attention on the growth of jaw and palate For extensive background information see references

RÉSUME

Le crane de l adulte avec une fente de la face non opérée presente des autres anomalies specifiques pour le type de la fente La creation artificielle d une fente pareille chez le lapin au cours de la quatrieme semaine après la naissance donne des anomalies semblables Quelques techniques chirurgicales (Veau l ostéoplastie primaire) pour corriger une fente de la face sont comparees au point de vue de leur effet sur le developpement ulterieur du crane du lapin L importance de la chirurgie experimentale avant d introduire des nouvelles techniques chirurgicales dans le domaine du crâne et de la face encore en developpement est accentuee

ZUSAMMENFASSUNG

Schadel von erwachsenen unbehandelten Spaltträgern haben weitere fur die Art der Spalte charakteristische Anomalien In einem tierexperimentellen Versuch haben wir festgestellt daβ in Kaninchen mit in der vierten Lebenswoche chirurgisch gesetzten Spalten gleichartige Schädelanomalien entstehen Zweitens wurden Untersuchungen ausgeführt zur Beurteilung des Einflusses verschiedener operativen Maβnahmen (Veau primare Osteoplastik) zur Korrektur der chirurgisch gesetzten Spalten und mit Hinblick auf das spatere Wachstum des Schadels junger Kaninchen Die Bedeutung experimenteller Untersuchungen vor der Anwendung neuer chirurgischen Methoden im Bereich des Gesichtsschädels wird hervorgehoben

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DISCUSSION

D F Harrison Does the lateral incisor present a problem in the construction of the experimental cleft? Is there any difficulty in keeping the clefts open and have any of them become infected resulting in bony loss

S R Wallstein Die experimentellen Verformungen bei diesen Tierversuchen haben infolge der Gestalt der Oberkiefer der Tiere auch mich sehr beeindruckt Die Kieferchirurgen zweifeln heute selbst ob die Implantation eines Knochenspans in den Alveolarfortsatz – beurteilt am Spatresultat – eine ausreichend physiologische Methode ist Ein ORL Kollege bei uns Schweckendieck hat 1945–48 ein anderes Programme ausgearbeitet 1) Operativer Verschluss der Lippenspalte im Alter von 3–4 Wochen 2) Verschluss der Spalte im *weichen* Gaumen in 2 –3 – Lebensjahr 3) Verschluss der knöchernen Spalte mit 10–12 Jahren Der Verschluss der Weichteile der Lippe und dann des weichen Gaumens induziert im Knochenwachstum eine Regulation der Stellung des Zwischenkiefers und eine starke Verangung der Spalte im harten Gaumen die leicht verschlossen werden kann

B H Colman The clinical importance of your findings especially in respect of the other more widespread alterations in the various bony structures cannot be overstressed They have been very beautifully demonstrated and accurately measured in the experiment From the treatment viewpoint it is also necessary to remember however that the bony changes which have been demonstrated were by no means fixed in young patients and indeed were easily reversible Would you agree that continual upward pressure of the tongue into the palatal cleft (forward against the premaxilla in the case with bilateral cleft lip) was a significant factor to be considered in clinical management? Pre-operative orthodontic care designed to prevent such pressure seemed to indicate that this was important and undoubtedly such orthodontic care could produce a dramatic reduction in the severity of the deformity and thus make eventual surgical correction of the residual cleft much easier

M Portmann Si j ai bien compris il y a un manque de developpement tres important des maxillaires superieurs après traitement par ostéplastie Ce manque de developpement est identique à celui que l on obtient apres extraction du cartilage septal (sur des bebes lapines par exemple) Ma question est la suivante Ne pensez vous pas que l ostéoplastie bloque en fait le cartilage septal qui a son tour ne « pousse » pas en avant le developpement de la face?

Verwoerd (Reply) to Mr *Harrison* In animals with unilateral cleft lip and alveolus and unilateral cleft lip alveolus and palate the incisor on the cleft side is often lost because of destruction of its root In control experiments we found that extraction of one of the incisors did not influence to some extent the outgrowth of the upper jaw In some animals the surgically produced facial clefts were closed in part spontaneously during the second half of the experimental period Comparing animals with open and spontaneously closed facial clefts we could not find morphological and geometrical differences

To Mr *Wallstein* The results of our experiments so far are in favour of the conservative principles of Veau s method Nevertheless as this method does not result in normal skull development Therefore we are studying the effects of new "suture replacing" techniques on the later development of the skull of rabbits with facial clefts

To Mr *Colman* and Mr *Portmann* In this paper the

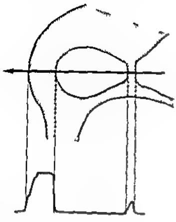

Fig 1

In the present densitometric investigations we were able to prove our histological findings, i e that bone apposition takes place on both sides of the separating wall This apposition can be seen even better on densitometric curves which make evident not only the bony substance but also the osteoid The osteoid can namely be demonstrated according to Bochatirchuk by microradiography and hence also in densitometric curve The densitometric curves of the separating wall, cochlear cavity of the basal coil and the cochlear wall differ in the various age groups

ne part of the basal coil protrudes into the panic cavity as the promontory and the figuration of its wall will thus depend throughout life not only upon the changes in the cochlear canal but also upon those in the tympanic cavity Measuring of the width of the cochlear wall in this region will therefore not give us real and precise values It means that the tympanic or outer part of the densitometric curve will vary according to the circumstances in the tympanic cavity The inner or cochlear part of the curve will correspond to the changes in the cochlear canal This inner part of the curve may be perpendicular or it may slope down so that the position of its lower end in relation to its upper end, expressed in centimetres can vary from 0 cm to 3 cm In young persons it is perpendicular and becomes more oblique with age This part of the curve always remains smooth, however, probably due to movements of the labyrinthic fluid The tympanic part of the curve sometimes shows indentations corresponding to the situation in the tympanic cavity The top of the curve also has indentations which might correspond to the interglobular spaces (Krmpotic & Nikolić, 1970) The oblique aspect of the inner part of the curve indicating the thickness of the cochlear wall is, in our opinion, a sign that bone apposition has taken place also in this region of the labyrinth and that the cavity of the cochlear canal can be narrowed from this side too

The height of the cochlear curve indicates the mineral contents in centimetres It should be stressed here that 3 5 cm on the densitometric curve corresponds to 1 mm of the shadow on the submicroscopic radiography The width of the cochlear wall in the densitometric curve varies in young individuals from 8 5 cm to 18 cm (11 58 cm, on average) and in the elderly from 8 6 cm to 14 5 cm (10 62 cm, on average) The height of this wall varies in young individuals from 9 2 cm to 19 cm (12 64 cm, on average) and in elderly individuals from 6 3 cm to 17 8 cm (11 99 cm, on average)

The part of the curve corresponding to the cavity of the cochlear canal is a straight line The diameter of the cochlear cavity varies in young individuals from 9 cm to 13 3 cm (10 86 cm, on average) and in elderly individuals from 7 5 cm to 13 1 cm (10 49 cm, on average)

The curve of the separating wall is lower than that of the cochlear wall and its shape also changes in different life periods In young persons the curve is high and steep on both sides with a narrow base and one constant notch corresponding to the cavity containing the spiral ganglion In young individuals this wall has a high mineral content in spite of its relative narrowness The actual indentation on the curve appears later on both sides of the curve, the one facing the cochlear cavity and also on the one facing the internal auditory meatus The indentation corresponds

to the apposition which takes place on both sides of the separating wall The indentation on the top of the curve corresponds to the nerve channels

The base of this curve becomes larger with age, while its height is reduced, which means that the reduction in mineral content parallels the ageing process The length of the base of the curve of the separating wall varies in young individuals from 1 8 to 8 5 cm (4 49 cm, on average) and in elderly individuals from 2 1 cm to 8 5 cm (4 66 cm, on average)

The height of the curve varies in young individuals from 3 6 cm to 10 7 cm (7 16 cm, on average) and in elderly individuals from 0 5 cm to 9 4 cm (5 32 cm, on average)

CONCLUSION

On the basis of our present investigations we can draw the following conclusions The width of the cochlear cavity shows a slight reduction with ageing It is the increasing thickness of the separating wall and the bone appositions on the side of the cochlear wall facing the cavity which are responsible for this reduction

The separating wall grows in thickness with age, while the mineral contents are reduced significantly Present investigations in our department are directed (Štern–Padovan) towards the changes in position of the cochlea and adjoining structures during life, as has already been mentioned by Alexander

At the same time we are trying to demonstrate the differing density of the bone by using Agfa contour film and Agfa contour developer to prepare the aequidensites For colour photographs we shall use colour paper and filters of Fotokemika Zagreb

RÉSUMÉ

La quantite de minéraux dans la capsule labyrinthique et dans la cloison vers le conduit auditif interne a ete etudiee en differentes periodes de la vie par la densitometrie La quantite de mineraux est reduite dans la capsule et dans la cloison en depit de l accroissement de la cloison par apposition osseuse

ZUSAMMENFASSUNG

Die Quantitat der Minerale in der Labyrinthkapsel der Basalwindung und der Scheidewand zwischen derselben und dem inneren Gehorgang in verschiedenen Lebensperioden wurde mittels der Densitometrie bestimmt Die Menge der Minerale nimmt sowohl in der Labyrinthkapsel als auch in der Scheidewand ab obwohl sich die Scheidewand durch Knochenapposition in der Regel verdickt

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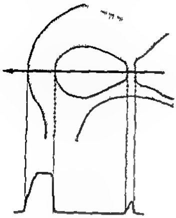

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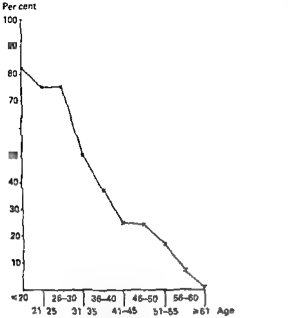


Fig 1 Persons with normal hearing in different age groups

From the values recorded in this examination, two interesting aspects will be reported One concerns the relation between social adjustment among the workers and the hearing-loss, and the other is the relation between discrimination measured without and with lip reading, where TV is used for discrimination

As regards workers followed up in different ways, but especially from a socio medical point of view where they had to answer a questionnaire which had a special reference to hearing handicap (Fig 2) It could be shown that even in the groups where the hearing loss was great, a surprisingly good social adaptiveness was achieved, especially on comparison with a material from a big city This is in fact not particularly extraordinary and can of course, be easily explained by the fact that the material consists of a very homogeneous group of workers who have been working together for many years They not only work together but they also meet outside work They have the same interests, which must make it easier to understand daily conversation

Confidentially

Questionnary to hearing examination in the Country of Vasterbotten

Personal identification
Name
Company
Hearing aid ☐ Yes ☐ No

1 Have you visited a doctor because of ear trouble or hearing loss?
☐ Yes cause Year
☐ No

2 Have you any symptoms of disease?
☐ Never
☐ Sometimes
☐ Often
Which

3 How many days have you been out of work because of disease 1973?
days because of

9 Can you understand what is said speaking to 1 person in a noisy place?

a
☐ without difficulty
☐ with certain difficulty
☐ with difficulty
☐ with great difficulty
☐ not at all

10 Can you understand what is said speaking to several persons in a relatively quiet place?

a

11 Can you understand what is said speaking to several persons in a noisy place?

a

12 Can you understand what is said in telephone?

a

13 Can you understand what is said looking at television?

a

Fig 2 Two parts of the questionnaire used in judging the social handicap of workers with hearing loss

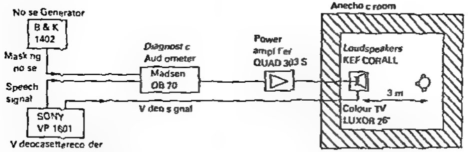
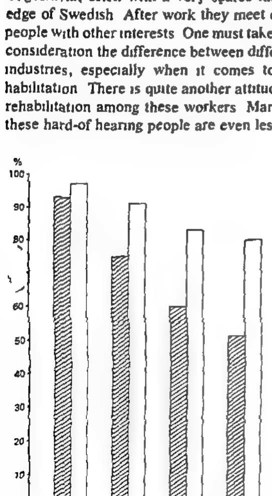


Fig 3 Test equipment for examination using TV as an audiovisual aid for discrimination

In the big shipbuilding yards in Gothenburg, for instance, the majority of the workers are from foreign countries, such as Italy and Yugoslavia, often with a very sparse knowledge of Swedish After work they meet other people with other interests One must take into consideration the difference between different industries, especially when it comes to rehabilitation There is quite another attitude to rehabilitation among these workers Many of these hard-of hearing people are even less interested in hearing aids than in a good amplifier for the TV or for the telephone

The other part of our investigation was a comparison between an audiovisual and an ordinary audiological examination of normal people and people with hearing loss TV is used and the results are compared with those reached using the ordinary speech audiometry (Fig 3) We compared the results from different hearing groups We also compared them with normal persons where disturbing noise is used The signal (*S*) was given at 65 dB and the difference between the signal and the noise



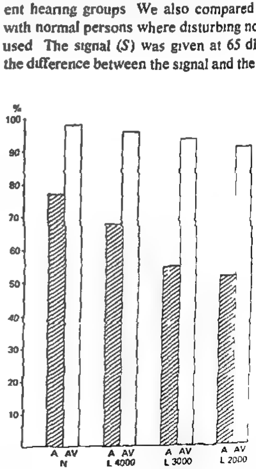


Fig 5 Same as Fig 4 The *S/N* however is 10 dB

(*N*) was varied using 15, 10 and 5 dB Details of this investigation will be published later In noisy surroundings even normal persons had a much better discrimination when they were tested audiovisually The results measured in different hearing levels showed a great improvement in the understanding of words and sentences when audiovisual testing was used (Figs 4, 5)

It was also possible to show that nearly every normal person even in a very noisy room could easily understand the spoken word if he was allowed to lip read at the same time This shows that everybody is more or less able to lip read, a fact that explains why nearly all persons are disturbed when sound and picture are not synchronised on the TV

The conclusion drawn from this special part of our research is that a person with a pronounced hearing loss can still easily be reached audiovisually This may be of great importance when judging the steps taken in safety matters *Orders should not only be* given by word of mouth but be combined with visual signs

ACKNOWLEDGEMENT

This paper is a teamwork based on the co-operation with Hans Danielsson Hans Granberg Roland Tengling and Stig Wiklund

RÉSUMÉ

Une enquête approfondie des scieries et des industries de masse à papier montre que meme les travailleurs ayant une surdité prononcee n ont pas de handicap social presume

ZUSAMMENFASSUNG

Eine sorgfältige Untersuchung von Arbeitern der Sagewerks und Papiermasseindustrie zeigte daß auch Arbeiter mit schweren Gehorschaden kein ausgesprochenes soziales Handicap hatten

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DISCUSSION

G Lidén It was interesting to hear that your patients seemed to be very little handicapped in spite of their hearing impairments A severe noise induced hearing loss usually gives rather good discrimination score in quiet but bad results in every-day background noise I think that your patients were unusually tolerant

F Escher Could you observe a higher amount of sudden loss of hearing?

J Tonndorf I have two questions one technical the other general 1) What type of interfering noise did you employ? 2) Do you see a possibility to employ your measurement technique for quantitizing the social handicap of workers with noise induced hearing loss? What I have in mind would be a combined index expressing the hearing ability in quiet in noise and aided by visual clues and perhaps a combination with Lidén s binaural test I do not believe in workers rating themselves subjectively This would be important because in compensation cases the judge wants to have numbers Complaint that cannot be quantified are never recognized at least in the US

H Diamant (Reply) to Mr *Lidén* I agree that it seems rather surprising that so relatively few complain of their hearing loss As I said however it is a very stable body of workers who know each other well and who meet even after work They are easily satisfied They are also very much depending on their work as their factory is the only big industry in the community I agree too that the testing of hearing should not be done in a camera silenta [illegible] few and it is therefore not possible to draw any conclusion concerning a higher morbidity of sudden deafness in workers of noise-industries

To Mr *Tonndorf* The disturbing noise is wide band white noise We have tried more natural noise like sounds from a cocktail party or from the traffic This noise is however not reliable as it fluctuates too much It is very true that you must give accurate and reproducable figures of the hearing handicap This is not easy and in Sweden so far not an absolute must I think however that with a lot of thinking and much work one can base an estimation on the figures we have available

Acta Otolaryngol 81:264–269, 1976

POSTAURICULAR (12 MSEC LATENCY) RESPONSES TO ACOUSTIC STIMULI IN PATIENTS WITH PERIPHERAL, FACIAL NERVE PALSY[1]

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Abstract The postauricular (12 msec latency) response to a loud click stimulation was investigated in 23 patients with facial nerve palsy Of these patients 21 had an intracranial cause for the facial palsy and 2 had an extracranial cause Only in those with the extracranial facial nerve damage could the response be obtained from both sides *When the nerve was damaged intracranially there* was no response on the paralysed side This seems to indicate that the posterior auricular muscle is the effector of the presumed sonomotor reflex arc involving the cochlea cochlear nerve and nuclei undetermined brainstem pathway facial motor nucleus facial nerve and the muscles supplied by it

The question regarding the site of the generators of the postauricular (12 msec latency) responses to acoustic stimuli, described by Kiang et al (1963), has not been completely solved The most probable hypothesis is that this response is one of the sonomotor reflexes and involves the cochlea, cochlear nerve and nuclei, undetermined brainstem pathway, facial motor nucleus, facial nerve and the muscles supplied by it It might be expected that stimulation of one cochlea would evoke a response from the postauricular area on both sides, providing the reflex arc is intact

Even though it is a simple and quick technique and probably provides information about auditory brainstem pathway the clinical usefulness of this method is somewhat doubtful because of the great intersubject variability of results There is as yet, no satisfactory explanation for this fact and the postauricular response has not attracted sustained interest in most centres (e g Cody & Bickford, 1969, Cody, 1974, Yoshie & Okudaira, 1969, Yoshie, 1974) Only in Douek's (Douek et al, 1973) laboratory is this method—referred to by them as the "crossed acoustic response" —employed routinely as a method of objective audiometry in children

In the Otolaryngological Department of the Warsaw Medical Academy, investigation of the postauricular responses is continued on a research rather than on a clinical diagnostic basis

In patients with unilateral or bilateral hearing loss, acoustical stimulation of the deaf ear does not evoke the postauricular response The aim of this investigation was to examine the postauricular response in patients with lesions at the end of the presumed reflex arc

TECHNIQUE AND METHOD

256 wideband clicks of 110 dB peak SPL, presented at a rate of 10/sec and transduced by TDH-39 earphones were used to evoke the postauricular response It was recorded as the potential difference between needle EEG electrodes inserted hypodermically in the post auricular sulcus in line with the top of the external acoustic meatus on both sides and on the vertex The electrodes were connected as

[1] This investigation was supported by a grant (373/VI) from the Polish Academy of Sciences

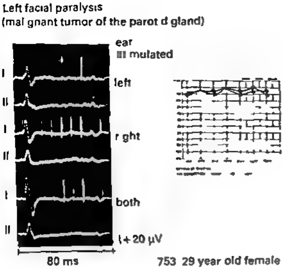

Fig. 1. The responses are recorded on both sides (channels I and II).

follows: left postauricular–vertex: channel I, right postauricular–vertex: channel II. The negativity on the mastoid was marked upwards. The disc ground electrode was placed on the forehead. The subject was seated in an armchair, holding his head down. The room was electrically shielded, but not sound proof (the noise level was 37–40 dB A). The recording bandwidth was 1.6–500 Hz. The responses were amplified and averaged as described elsewhere (Bochenek & Bochenek 1975). In the majority of cases promontory—ear lobe electrocochleography *ad modum* Portmann–Aran was also performed.

SUBJECTS

Twenty three unsedated patients (11 females and 12 males, aged 16–60) with unilateral peripheral facial nerve palsy were examined. In 21 the nerve was damaged intracranially (group A), in 2 extracranially (group B).

In group A the disease was diagnosed as Bell's palsy (9 cases), skull trauma (4 cases), otitis media (4 cases), acoustic neuroma (2 cases), cholesteatoma of the temporal bone (1 case), VIII nerve neurectomy (1 case).

In group B the facial nerve paralysis was caused by a malignant tumor of the parotid gland in both cases.

In 13 patients the left facial nerve was paralysed, in 10 the right.

RESULTS

Only in patients with extracranial facial nerve damage could the postauricular response be recorded from both sides. In no patients with

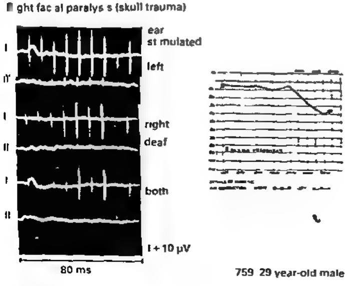

Fig. 2. The response is recorded only on the normal (left) side (channel I). The stimulation of the deaf (right) ear does not evoke the postauricular responses (channels I and II).

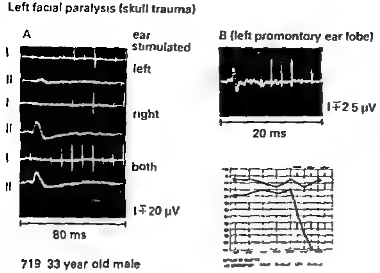

Fig 3 The responses are recorded on the normal (right) side only (channel II)

an intracranial lesion of the VII nerve was there any response from the paralysed side

The responses from the normal side do not differ from those recorded in healthy people A great intersubject variability of amplitudes, but rather small differences in the latency of the responses, were found

In a patient with delayed and temporary mild facial nerve palsy following VIII nerve neurectomy repeated testing of the post auricular responses was performed, as shown in Fig 5 before surgery (A) just after VII nerve palsy appeared (B) and after full clinical recovery (C) The reappearance of the post auricular response on the previously paralysed side may be seen

DISCUSSION

The results of recordings using the above mentioned placing of the electrodes on the postauricular area seem to indicate that the effector of the presumed reflex arc can be identified as the posterior auricular muscle

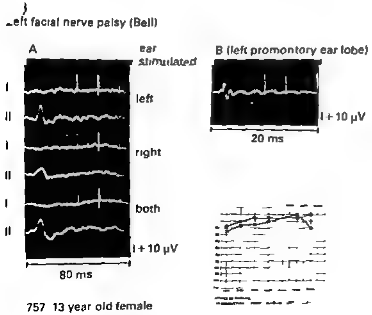

Fig 4 The responses are recorded on the normal (right) side only (channel II)

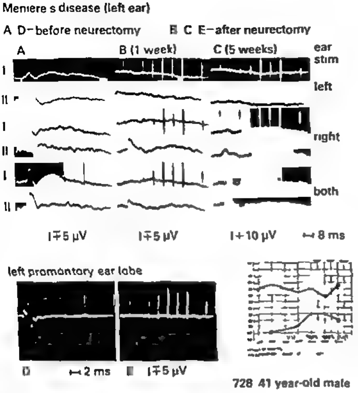

Fig 5 Before VIII nerve neurectomy the postauricular responses were recorded on both sides (A) After the postoperative VII nerve palsy appeared (B) there was no re sponse on the left side (channel I) After recovery this response reappears (C) The stimulation of the left ear—deaf after neurectomy—does not evoke the postauric ular responses (B C channels I and II)

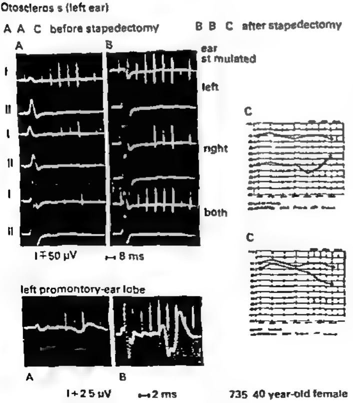

Fig 6 The sectioning of the tendon of the stapedius muscle did not abolish the post auricular response on the ipsilateral side (B channel I)

The arguments in favour of it are (i) bilateral, postauricular responses were obtained only in patients with extracranial facial nerve damage, peripheral to the stylomastoid foramen which implies that such damage has occurred after the posterior auricular nerve has left the facial nerve, (ii) in patients with intracranial facial nerve paralysis (central to the stylomastoid foramen), implying that the damage is before the posterior auricular nerve has left the facial nerve, there was no response from the paralysed side, (iii) because of the vicinity of the electrode, the possible influence of the middle ear muscle's activity—especially of the stapedius muscle—on the postauricular response must be borne in mind The recordings made in an otosclerotic patient after stapedectomy suggest that sectioning of the tendon of the stapedius muscle does not abolish the postauricular response on the ipsilateral side

It was not the intention of this study to compare the postauricular response with results of diagnostic tests usually employed in patients with facial nerve palsy Perhaps a more detailed analysis of the muscular response to acoustic stimulation—a kind of 'sonomyography'—could be of certain diagnostic and prognostic value in these cases

RÉSUMÉ

La reponse postauriculaire (12 msec de latence) evoquée par la stimulation sonore est—parait il—un des reflexes sonomoteurs Malgré sa grande variabilité elle presente aux audiologistes un interet tout particulier parce qu elle peut contenir les informations sur le trajet auditif dans le tronc cerébral Pour identifier l effecteur de l arc reflexe presumé on a examine les malades atteints de la paralysie intra ou extracranielle

ZUSAMMENFASSUNG

Die postaurikulare Antwort (von 12 msek Latenzzeit) auf einen lauten Reiz ('click') wurde bei 23 Kranken mit peripherer Fazialisparese untersucht Von diesen Kranken lag bei 21 die Ursache von der Parese intrakranial und bei 2 extrakranial vor Nur bei Patienten mit extrakranialer Fazialislasion konnte man die Antwort auf beiden Seiten bekommen In Fallen in welchen der Nerv intrakranial beschadigt war wurde keine Antwort auf der gelahmten Seite beobachtet Infolgedessen scheint es uns daß der M auricularis posterior den Effektor vom vermutlichen sonomotorischen Reflexbogen darstellt welcher von der Cochlea des N cochlearis der Cochleariskerne einer unbestimmten Hirnstammbahn des motorischen Fazialiskernes des N facialis und der von ihm versorgten Muskeln zusammengesetzt ist

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DISCUSSION

Mr Suzuki From the audiological point of view the most serious disadvantage of this response is the inconsistency or variability on its appearance In my experience about 40% of the adult subjects with normal hearing show clear responses with stimulus 10 to 30 dB above subjective thresholds But another 30% needed 40 to [illegible] dB stimulation and in the remaining 30% the response was detectable with stimulus above 70 dB or could not be detected at all even with the maximum intensity 90 dB Such an individual variability is a very serious problem for applying the response as an index of the objective audiometry However we should overcome this problem because the response is a very important and easily recordable one as you have stated

Mr Beagly Do you think that the late response observed sometimes in some cases during transtympanic electro-cochleography using a 20 ms analysis time is an example of the postauricular response? The slide I put up showed such a response which was abolished with Succinylcholine but recovered after a few minutes This indicated that it was muscular in origin I think that it is probably from the postauricular muscle and is picked up from the electrode which is situated on the earlobe

Z Bochenek (Reply) to Mr *Suzuki* In our previous paper we reported our findings concerning *threshold* measurements of the postauricular response in normal

aring subjects and in patients with different kind of aring loss This threshold was from 10 to 50 dB Be use of great intersubject variability of the postauricular sponse we think that it can be used as a clinical ol for objective evaluation of hearing only together ith other tests of electric response audiometry such as ectrocochleography and vertex responses In this report patients with facial nerve palsy served as a model to examine the arc of the presumed sonomotor reflex

To Mr *Beagly* In our promotory-ear lobe recordings using 20 msec analysis time in some patients a component of about 12 msec latency could be seen It seems it is a myogenic response generated by posterior auricular muscle

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EFFECTS OF ACOUSTIC TRAUMA ON CORTI'S GANGLION

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Abstract. The ultrastructural features of Corti's ganglion cells were studied in guinea pigs subjected to a wide band plain 100 dB SPL white noise (12 hr/day for 30 days). Regression was primarily observed in cells belonging to the 2nd turn and took the form of detachment of the membrane from the myelin sheath and cytoplasmic retraction to the point of destruction of the entire cell. Intensely osmiophilic myelin bodies were frequent in Schwann cells and in gangliar cells and their sheath. Multivesicular bodies were also common. A description of the regression noted in the myelin fibres is given. Attention is drawn to the fact that regression was not observed in sheathless fibres and cells: the latter formed about 5% of the ganglion population.

The submicroscopic features of the cells of Corti's ganglion have been described in a number of animals: the rat (Rosenbluth, 1962, Ross & Burkel, 1973), the cat (Spoendlin, 1971, 1972), the guinea pig (Suzuki et al., 1963, Nishimura et al., 1965, Thomsen, 1966, Awataguchi et al., 1967, Kellerhals et al., 1967, Reinecke, 1967), and the rabbit (Suzuki et al., 1963). In line with the light-microscope data, these findings have shown that these cells are opposito polar bipolar. The pyrenophore is thus crossed by the nervous impulse. To meet this requirement and at the same time maintain the metabolic exchange with the interstitium, it is covered by a myelin sheath whose submicroscopic features distinguish it from that of the nerve fibres.

The lamellae of the nerve fibre form a closely packed sheath (compact myelin), whereas in the case of the pyrenophore they are either distinct, and may contain cytoplasmic organules (dissociated myelin), or loosely packed (semicompact myelin).

The existence of a contingent of cells not covered by a myelin sheath has also been demonstrated. More precisely, these are cells surrounded by a single layer of cytoplasm of the satellite cell. These cells are particularly rich in cytoplasmic microtubules and microfilaments (Suzuki et al., 1963, Kellerhals et al., 1967, Reinecke, 1967, Nishimura et al., 1965, Thomsen, 1966, Awataguchi et al., 1967, Spoendlin, 1971, 1972, Ross & Burkel, 1973). Recently Spoendlin (1973) has described another type of ganglion cell which closely resembles the cell with myelin sheath but is not itself myelinated.

It is not certain what function these cells have. Spoendlin (1971) suggests that they are responsible for the afferent innervation of the ciliated external cells of Corti's organ, while Ross & Burkel (1973) consider they are multi

Fig. 1. Concentric lamellar myelin structure (*l*) containing intensely osmiophilic clumpings. This structure lies in a space (*c*) containing shapeless residues and created by detachment of the cell membrane from the myelin cover. An inclusion (*b*) can be seen between the lamellae of the sheath.

Fig. 2. Osmiophilic dense body wrapped in a formation whose periodic structure is still partly maintained (*a*). The gangliar cell sheath has lost its uniformity and is dissociated. Two giant mitochondria belonging to the Schwann cell can be seen (*b*).

Fig. 3. Lamellar and intensely osmiophilic amorphous structures in the Schwann cell.

This research was supported by a grant from C.N.R.

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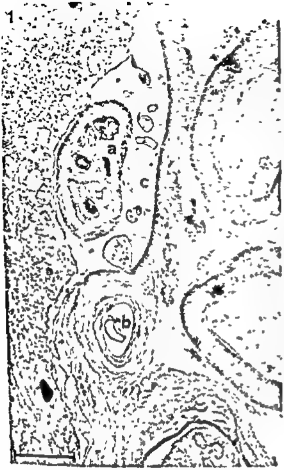

2


3


polar cells belonging to the parasympathetic autonomous system

Changes in cells of Corti's ganglion induced by a prolonged acoustic stimulus have been examined microscopically and ultrastructurally Hamberger & Hyden (1945), Hammer (1956), Beck & Beickert (1958), Beck & Michler (1960), Thomsen & Pakkenberg (1962), Vinnikov & Titova (1963), Kon (1964), Wüstenfeld & Halbfas (1965), and Wustenfeld & Schilling (1966) have shown both quantitative and qualitative cytochemical and histochemical changes, while Wicke & Firbas (1970) noted a significant reduction in the number of ganglıar cells per surface unit

Electron microscope studies by Awataguchi et al (1965) and Kellerhals et al (1967) have been reported Guinea pigs were subjected to the firing of 160–200 blanks from a pistol at a distance of 10–20 cm in 2–3 hours Awataguchi et al (1965) noted swelling of the mitochondria, with deformation and disappearance of the cristae, dilatation of the cisternae of Golgi's complex, and vesicles in the myelin sheath Kellerhals et al (1967) observed "Riesen" mitochondria in the satellite cells, vacuolised pigmented bodies, myelin bodies, and cytoplasmic inclusions wrapped in myelin groups expressed the view that the re ion observed involved cells with either thed or unprotected pyrenophores

MATERIALS AND METHODS

Fifteen male guinea pigs weighing between 300 and 400 g (plus 3 controls) with a distinct, active Preyer reflex were exposed for 12 hr/day and for 30 consecutive days to a wide band, plain 100 dB SPL white noise generated by an ELIT 815 audiometer Intensity was measured with a Bruel & Kjaer 1612 phonometer at the grille of an Isonetta Kompaktbox loudspeaker connected to a bar resting on the top edge of the 50×50×30 cm box containing the animals

Three animals died during the experiment The remainder were sacrificed in groups of three at a time, 7, 15, 22 & 30 days after the experiment

Under ether, each animal was perfused by insertion into the aorta of the cannula connected to a gravity operated mechanism The circulating mass was then washed for 1 min with an 0 9% NaCl solution in pH 7 4 phosphate buffer After fixation with a mixture of 2% paraformaldehyde and 3% glutaraldehyde in the buffer, perfusion was continued for 60 min

After removal of the bulla, lamina spiralis and Corti's organ, the modiolous was kept for a further 60 min in the same mixture and then washed for 4 hours with an 8% dextrose solution in pH 7 4 phosphate buffer, followed by post fixation in 2% osmium tetraoxide for 1 hour After embedding in Araldite (Ciba), sections were cut with LKB and Reichert microtomes and stained for light microscopy according to Richardson's method, prior to examination with Siemens Elmiskop IA and 102 electron microscopes

RESULTS

Two types of ganglıar cells were observed in the controls, mostly covered with a partly dissociated, partly semicompact myelin sheath In some cells, the semicompact layers were behind the cell body, in others, the dissociated layers were on the inside After the initial segment of the nerve fibre these features gave way to the classic compact myelin sheath

Sheathless cells were also noted here and there These were wrapped in a thin cytoplasmic layer supplied by a satellite cell Their frequency was no higher than 5% Their nucleus was often eccentric, their cytoplasm was

Fig 4 Myelin sheath of a ganglıar cell whose lamellae are clearly dissociated and are enveloping a myelin body

Fig 5 Ganglıar cell with distinctly dissociated myelin sheath (*a*) The membrane is detached from its sheath in (*b*)

Fig 6 Retraction of cell body with extensive detachment of the sheath The cytoplasm contains dense bodies probably lysosomes


4
5
b
b

crossed by many neurotubules and nerve tubules and filaments and they were poorly supplied with smooth and rough surfaced ER Sheathed and sheathless cells had varying amounts of free ribosomes numerous mitochondria and occasional probably lysosomial thick bodies Unsheathed fibres were observed among the two cell types described and those with compact myelin

Regression was particularly evident in gangliar cells situated between the end of the 1st and the beginning of the 3rd turn Its chronological progress in relation to the date of sacrifice could not be established since the signs to be described coexisted in animals from each group they were however much more numerous and more evident on the 30th day

Mitochondria were reduced in number and showed signs of regression on the 30th day only This fact deserves attention along with the very slight difference noted between the morphology clumping and ultrastructural features of the ER and ribosomes in the treated animals and the controls

Most cells in the 2nd turn showed a remarkable increase in lysosomes Some of these particles contained variously opaque inclu ns and were often intermingled with multi icular bodies which were probably due to confluence and clumping of lysosomes and with microvacuoles and cytoplasmic dense bodies

Myelin bodies of various shapes and sizes were noted between the cytoplasmic membrane and the inner layers of the myelin sheath of these cells These bodies were intensely osmiophilic and had a distinctly concentric lamellar structure though there were some whose pre existing structure was no longer marked by the presence of elements endowed with any particular degree of periodicity (Fig 1) Similar myelin bodies were noted in the Schwann cell cytoplasm This often contained giant Riesen type mitochondria (Fig 2) Such bodies were also observed on a number of occasions in the myelin sheath of the ganglar cells particularly between the inner layers and in dissociated myelin areas (Figs 3 4)

Changes in the shape of the nucleus and signs of retraction heralding destruction and disappearance of the cell itself were more common in animals subjected to longer periods of stimulation and may be thus seen as a more advanced stage The chromatin was often powdery In some cells the nucleolus was broken up into lumps up against the membrane in others it was unrecognisable even in serial sections while entrenchments and festoons in the nuclear border pointed to its sub-division and fragmentation

The membrane of some cells was only stripped from the myelin sheath at various points on the circumference (Fig 5) in other cells retraction had led to the formation of large gaps that often contained shapeless residues (Fig 6) crossed by thin shoots that were probably derived from the innermost layer of the sheath and connected the cell to its original cover

In some instances the entire cell body had dissolved into a series of wide vacuoles that occasionally contained shapeless residues (Fig 7)

The changes noted in the implantation cone and in the nerve fibres were the same as those in the cell body Where the myelin sheath was compact these changes consisted mainly of stripping of the outer coat of the axon from the innermost layer of the sheath This was presumably the prelude to complete retraction and disappearance of the axon Myelin bodies could sometimes be seen in the axon and in the Schwann cell cytoplasm though less frequently than in the ganglar cells and their satellites

No signs of regression were noted in the sheathless ganglar cells and in their nerve fibres (Fig 8)

CONCLUSIONS

It is clear from these findings that protracted acoustic stimulation can cause severe damage

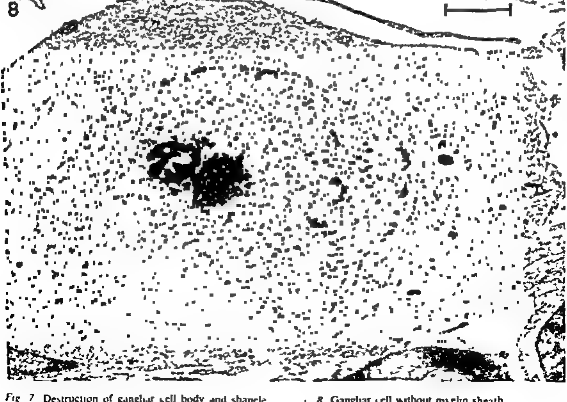

Fig 7 Destruction of ganglia cell body and shapele residues in the place it formerly occupied

‹ *8* Ganglia cell without myelin sheath zression are visible

crossed by many neurotubules and nerve tubules and filaments, and they were poorly supplied with smooth- and rough surfaced ER Sheathed and sheathless cells had varying amounts of free ribosomes, numerous mitochondria, and occasional, probably lysosomial, thick bodies Unsheathed fibres were observed among the two cell types described and those with compact myelin

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DISCUSSION

H Spoendlin The changes you describe affecting essentially the myeline sheath of type I cells correspond to changes due to retrograde degeneration after changes to the organ of Corti We found such changes only secondary to changes of the organ of Corti but never as a significant primary reaction of the spiral ganglion cells Did you evaluate the organ of Corti in your cases? Such alterations in the spiral ganglion cells can occur in a scattered fashion in the normal adult and especially older animal It would therefore be important to have quantitative information of the cells affected and to know the age of the animal

M Portmann Lorsque je travaillais les fibres efferantes j avais constate leur integrite dans le ganglion de Corti sur le ganglion spiral Avez vous etudie au microscope electronique egalement le faisceau spiral efferant et avez vous vu des modifications pathologiques? (Spoendlin ayant depuis mes recherches trouve des anomalies dans ce faisceau apres trauma acoustique)

G Rossi (Reponse) A Mr *Spoendli* n En reponse a la première question que vous m avez pose je voudrais vous dire que l étude n a porte que sur le ganglion de Corti sans prendre en considération la correlation avec l organe de Corti Cette étude sera l'objet d'une recherche ulterieure Dans notre recherche nous avons essaye d etablir si la stimulation acoustique dommageait les cellules ganglionnaires et de determiner la progression de ces lésions

Pour avoir des donnés quantitatifs il est necessaire de tuer les animaux quelque mois apres la fin de la recherche Je pense que pour quelque lesion parmi celles que je vous ai presentees soit encore possible une « restituto in integrum » mais je pense aussi qu il y a une progression des lésions qui emporte la destruction de la cellule et cette destruction peut se completer en quelque mois Nous avons sacrifie nos animaux au 30ieme jour apres le debut de l'experience les donnees quantitatives dans ces conditions ont peu de signification

La recherche à eté executée en employant des animaux de 300–350 g qui avait a peu pres 4 mois

A Mr *Portmann* Je ne peu pas vous donner des renseignements à propos de fibres efferentes parçe que nous nous n avons pas pose cette question Je voudrais seulement vous dire que dans une autre expérience executée il y-a presque dix ans par le microscope optique j'avais detruit par le bruit l'organe et le ganglion de Corti du cobaye mais les fibres efferentes restaient normales

Acta Otolaryngol 81 278–282, 1976

MEASUREMENT OF OXYGEN TENSION IN HUMAN PERILYMPH

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Abstract The oxygen tension of the human perilymph has been determined without complications by using a technique based upon the polarographic principle Carbon dioxide produced the most marked increase in oxygen tension in the human perilymph No change in the perilymphatic PO_2 was observed after administration of eupaverine and low molecular weight dextran 5% CO_2–95% O_2 has been found to be the gas mixture producing the largest increase in oxygen tension in the human perilymph without side effects In order to achieve maximal oxygenation of the perilymph the therapeutic administration of *5% CO_2–95% O_2 should continue for at least 30* minutes At this preliminary stage it is not possible to assess the accuracy of the presented technique in determining the presence of a vascular disorder of the inner ear in patients

Changes in cochlear blood flow in response to vasodilating drugs and related agents were [illegible]ed in the guinea pig by Morimitsu (1960) [illegible] Suga & Snow (1969) by means of electrical [illegible] plethysmography According to [illegible] authors inhalation of carbon dioxide and amylnitrite increased cochlear blood flow while inhalation of pure oxygen decreased it Intravenous injections of papaverine, dipyridamole, bradykinin, kallidin, histamine, betahistine hydralazine a hypertonic glucose solution and a sodium and ▪ bicarbonate solution increased cochlear blood flow but reduced systemic blood pressure An extensive investigation of the effect of CO_2 on blood flow of otic tissue using radioactive microsphere techniques in mongrel dogs has been published recently by Pollock et al (1974) According to these authors carbon dioxide is the most potent otic *vasodilator To our knowledge* measurements of the oxygen tension in the human inner ear fluids have never been attempted before The aims of the present study were

(*a*) to develop a technique for the assessment of the oxygen tension in human perilymph
(*b*) to determine the factors increasing the oxygen tension in the human perilymphatic space, and
(*c*) to ascertain whether the measurement of *the oxygen tension in the perilymphatic* space may be of diagnostic value in circulatory disorders of the inner ear

MATERIAL AND METHOD

The method used for determination of the oxygen tension in the perilymph is based upon the polarographic principle A platinum and silver–silverchloride microelectrode with a tip of less than 100 µm in diameter has been constructed in collaboration with the Biomedical Engineering Institute of the Swiss Federal Institute of Technology and University of Zürich and tested in the laboratory of the ENT Department of the University of Zurich The electrode was introduced with a specially constructed micromanipulator through the fenestrated footplate of 5 patients suffering from otosclerosis and of 6 patients presenting with a severe hearing loss of the sudden type and who showed no signs of recovery after a week of conservative treatment The arterial PO_2 and Pco_2, as well as the blood pressure and *ECG were monitored continuously* during the experiments conducted under general anaesthesia

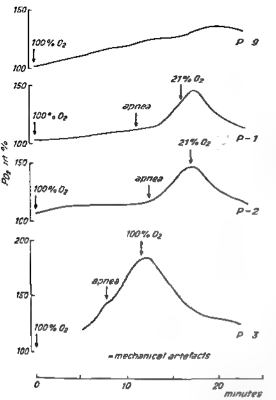

Fig 1 Oxygen tension of the human perilymph during inhalation of 100% oxygen Note the extent of the rapid increase in perilymphatic oxygen tension in apnea following inhalation of pure oxygen

RESULTS

During inhalation of pure oxygen for 15 to 20 minutes a very limited increase in CO_2 (less than 130% of the initial value) was measured (Fig 1) When the administration of pure oxygen was followed by 3 to 4 minutes of apnea, a rapid increase in the oxygen tension occurred in the perilymphatic space The observed increase in PO_2 correlated very closely with the P_{CO_2} but not with the PO_2 values recorded in the radial artery (Fig 2) These observations confirm therefore, the previously mentioned experimental investigations concerning the otic vasodilating properties of carbon dioxide

The inhalation of 5% CO and 95% O (Fig 3) in otosclerotic patients was followed by a very extensive increase of perilymphatic PO starting 3–6 minutes and reaching its maximum 16–33 minutes after the beginning of inhalation When administration of CO_2 was discontinued the perilymphatic PO, returned in 10–12 minutes to the initial values The curves shown for patients 4 and 5 are very comparable to those obtained by similar experiments in cats (Murata & Fisch, 1975) and can therefore be considered as the most representative In the experimental animal, however, the response to carbon dioxide is more rapid and the maximal perilymphatic PO_2 level is already reached within 5 minutes This difference must be related to the better vascular supply of the inner ear of the experimental animal, as compared with that in man In view of the longer time needed to reach the maximal perilymphatic oxygenation after inhalation of 5% CO_2 and 95% O_2 in the humans, the therapeutic administration of carbon dioxide (e g in acute hearing loss) should be continued for at least 30 minutes

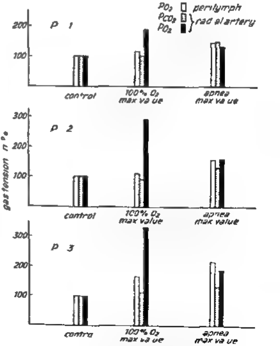

Fig 2 Comparison of perilymph and blood gas responses during inhalation of 100% O_2 and apnea Note the correlation between perilymphatic PO_2 and

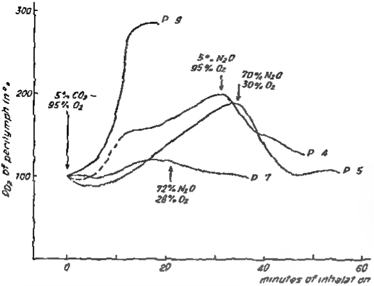

Fig 3 PO of perilymph during inhalation of 5% CO - 95% O in patients with otosclerosis The curves obtained for patients 4 and ▮ are very similar to those obtained by similar experiments in cats

In contrast to the 200% increase in perilymphatic oxygen tension following inhalation of 5% CO and 95% O no change in the perilymphatic PO_2 was observed after administration of eupaverine and low molecular weight dextran (Fig 4) Similar results were obtained in cats Of course, the negative influence of papaverine and low molecular weight dextran measured in normal inner ears could be different than that observed in pathological conditions However to date all recordings of the ymphatic oxygen tension in patients with deafness failed to reveal any response eupaverine and low molecular weight dextran

The measurements of perilymphatic PO in 4 patients presenting with sudden deafness are demonstrated in Fig 5 In 2 patients (P 5 and 10) no change in the PO could be recorded after inhalation of carbon dioxide In 2 other patients (P 8 and 11) the increase in the oxygen tension measured in the perilymph was similar to that recorded in patients with otosclerosis It is very tempting to conclude from these figures that 2 of our patients did indeed present with a vascular disorder of the inner ear at the time of measurement At this preliminary stage it is impossible to determine whether the normal response to carbon dioxide inhalation in 2 other patients was so be

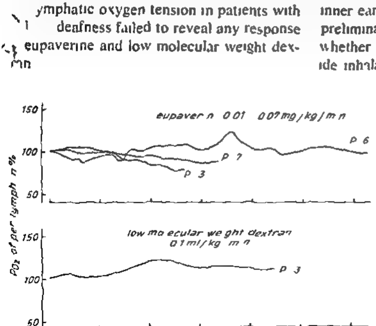

Fig 4 Response of oxygen tension of the human perilymph to vasodilating drugs

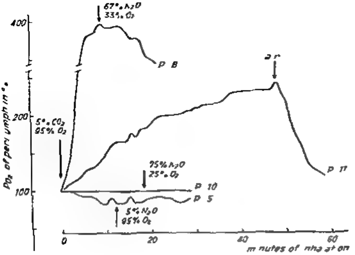

Fig. 5. PO_2 of perilymph during inhalation of 5% CO_2 – 95% O_2 in patients with sudden deafness. Note that in patients 5 and 10 no change in perilymphatic PO_2 could be recorded after inhalation of carbon dioxide.

cause they suffered from a transient vascular disturbance, or whether their sudden deafness was truly unrelated to any vascular problem.

In view of the above mentioned observations, repeated inhalations of 5% CO_2 – 95% O_2 for 30 minutes at one hour intervals have been used without side effects in our department for the treatment of sudden hearing losses of probable vascular origin. Although inhalation of 10% CO_2 – 90% O_2 has been found by Pollock et al. (1974) to give the largest increase in temporal blood flow in the experimental animal, a dangerous increase in arterial blood pressure was recorded during inhalation of this gas mixture in our patients. This effect never occurred with 5% CO_2 – 95% O_2. Thus caution should be exerted in administering higher concentrations of CO_2 in patients.

CONCLUSIONS

1. The oxygen tension of the human perilymph can be determined without complications using the presented technique which is based upon the polarographic principle.

2. Carbon dioxide produces the most marked increase in oxygen tension in the human perilymph.

3. The therapeutic administration of 5% CO_2 – 95% O_2 in patients should be continued for at least 30 minutes in order to reach maximal oxygenation in the inner ear.

4. At this preliminary stage it is not possible to assess the accuracy of the presented technique in determining the presence of a vascular disorder of the inner ear.

ACKNOWLEDGEMENT

We would like to express our gratitude to H. Maurer and W. Moller for their technical assistance.

RÉSUMÉ

La tension en oxygène de la périlymphe humaine a été déterminée par des microélectrodes introduites dans le vestibule à travers la platine de l'etrier. Les résultats obtenus après apnée, pendant l'inhalation de différentes concentrations d'oxygène, et après infusion de vasodilatateurs chez des malades souffrant d'otosclérose et de surdité brusque, sont présentés.

ZUSAMMENFASSUNG

Die Sauerstoffspannung der menschlichen Perilymphe wurde ... durch die fenestrierte ... platte ... Mikroelektrode unter Benutzung der polarographischen Methode gemessen. Die grösste Zunahme des perilymphatischen PO_2 wurde durch ... Inhalation von CO_2 beobachtet. Die Sauerstoff... der Perilymphe hat sich während der ... von Eupaverin und Dextran nicht ... therapeutische Zwecke hat sich ... von 5% igem CO_2 – 95% igem O_2 am besten bewährt. Um die maximale Oxygenation der Perilymphe zu erreichen, muss das Gasgemisch während wenigstens 30 Minuten eingeatmet

werden Wegen der geringen Zahl der bisher untersuchten Patienten ist es noch nicht möglich mit Sicherheit zu sagen ob die verwendete Technik für die Diagnostik vaskulärer Erkrankungen des Innenohres eingesetzt werden kann

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DISCUSSION

B M Johnstone 1) What is the stability of your electrodes? 2) The volume of perilymph in animals is much smaller than in man As diffusion time increases as the square of the distance could the slower response time in be due to the difference in diffusion time?

Wullstein My question concerns if the audio-effects as these experiments were made partly in ... We tried it in a few patients only clinically and ... seen nearly no answer

M Jacobs You reported about two cases without bettering the hearing loss after oxygen use We are nowadays treating inner-ear diseases by means of hyperbaric oxygen and intra arterial infusions of ATP with good results It will be published in the next months

H Spoendlin Can you explain the great individual variation of more than 100% in normal subjects?

K Murata (Reply) to Mr *Johnstone* 1) The tips of the pt electrode was slightly recessed and some electrical shock chambers were prepared in the amplifier in order to have a good stabilization The drift of the electrode current did not exceed more than 1-2%/hr 2) We agree that the bigger space of the human vestibule may play a role concerning the longer latency of diffusion rate of O_2 from the vessels in comparison to the latency found in animal experimentation

To Mrs *Wullstein* A comparative study on the treatment of sudden deafness with papaverine infusion and reomacrodex and with 5% carbon dioxide is on the way in the ENT Department of the University of Zurich The results have not yet been statistically analysed so that we are, for the moment unable to answer your question

To Mr *Jacobi* According to our animal experiments hyperbaric oxygenation with pure oxygen must be carefully applied for the clinical treatment since the tissue activity in the inner-ear is possibly endangered by a too high concentration of O_2 At any rate we would like to recommend to have 5% CO_2 in the inhalating mixture if you want to increase the perilymphatic O_2 as much as possible

To Mr *Spoendlin* The calibration of our electrode was performed immediately after the actual measurements in the perilymphatic space because of the problem of sterilization The absolute values measured after inhalation of 5% CO_2 and 95% O_2 were e g of 18 0 respectively 74 2 mmg in two cases of sudden deafness and of 29 4 mmg (air) for one case of otosclerosis The fact that in one of the four otosclerotic patients the response to CO_2 inhalation was not as good as in the remaining three is probably due to the initial difficulties we experienced in placing the microelectrode into the vestibule In all our later cases the curves obtained in otosclerotic patients are of the same shape as those recorded for patient 9 4 and 5

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CLINICAL APPLICATION OF AUDITORY NERVE RESPONSES RECORDED FROM THE EAR CANAL

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Abstract The practicality of recording auditory nerve responses in the routine practice of an otologist is demonstrated Such recordings are particularly useful when the course of treatment requires a distinction between central or peripheral disorders They are also helpful when it is difficult to obtain a valid audiogram The electrical recordings from the auditory nerve can in combination with standard audiological otological and neurological examinations present a more accurate picture of the patient's condition

Recently, there has been considerable interest in the use of electrical recordings from the cochlea and auditory nerve (electrocochleography) as a diagnostic tool While the clearer recordings can be made by placing an electrode directly onto the cochlea (Portmann et al , 1967, Yoshie, 1971, Eggermont & Odenthal, 1974), it is feasible to record auditory nerve potentials by means of less invasive techniques (Yoshie et al , 1967, Sohmer & Feinmesser, 1967, Salomon & Elberling, 1971, Moore, 1971, Keidel, 1971, Bochenek et al 1972, Cullen et al , 1972, Montandon et al , 1975*a*)

The present paper presents a personal evaluation by the author, a practicing otolaryngologist, of the usefulness of recording auditory nerve responses with ear canal electrodes (Montandon et al , 1975*b*) The following questions will be asked

1 In what types of cases are recordings indicated?
2 How common is the occurrence of such cases?
3 What are the economic factors in making these recordings?

This work was supported by a grant of the Academic Society of Geneva and by funds provided by the University of Geneva Medical School

METHODS

Generality

The author is practicing in a general hospital serving a population of approximately 400 000 The cases to be described were clinic and private patients referred because diagnostic problems had arisen that could not be resolved with otological examination and routine audiometric tests The recordings were usually performed by the author alone, although occasionally with assistance, especially if the subject was a child They were made either immediately following the first examination or later upon appointment The testing rooms were either standard ENT-examining offices or rooms set up for minor surgical procedures

Preparation

As described previously (Montandon et al 1975*a*), 3 electrodes are placed on the subject an active electrode in the ear canal a reference electrode on the ear lobe, and a ground electrode on the forehead All 3 electrodes are connected to a contact box held above the ear by an elastic headband With the subject lying on an examining table a binocular office microscope is used with a standard nasal

speculum to obtain a direct view of the posterior wall of the ear canal which is then carefully cleaned with cotton soaked in an ether-alcohol solution The active electrode, a gold foil disk 0 5 cm in diameter attached to a thin flexible gold wire, is covered with contact paste and placed with alligator forceps on the posterior wall near the tympanic membrane The end of the wire from the ear canal is gripped by clips attached to a connecting box

The portable stimulus generating and response recording device (Montandon et al, 1975*a*) is on a small wheeled table close to the patient's head The leads connecting the patient to the recording device are plugged in The resistance between the ear canal electrode and the reference electrode is measured and if it exceeds 30 Kohms, the electrodes are adjusted If necessary, the ear canal electrode can be removed and the skin cleaned again until a lower resistance is obtained

Test

The tester sits near the head of the subject The clicks are delivered through a plastic tube placed 4 cm from the subject's ear The recorded electrical responses are averaged and continuously displayed on the screen of the oscilloscope as a true average Thus the background electrical activity is gradually reduced and the responses become clearer as the clicks are repeated Whenever the average reaches a stable configuration the recording can be terminated and the resulting display photographed for a permanent record Click levels between approximately 70 and 110 dB SPL are available in 10 dB steps By putting the tube 1 cm from the ear canal it is also possible to reach a level of approximately 120 dB SPL After the test is completed the patient is disconnected from the machine and the canal electrode removed by pulling out the gold wire

Evaluation of responses

The response from a normal subject is typically a negative electrical deflection (N_1) of approximately 1 msec duration with an amplitude and latency that depends on the click level (Montandon et al, 1975*b*) The lowest click level that can produce a visually detectable average response is taken to be the "threshold" Under the present conditions most normal subjects show a detectable response at 70 dB SPL Thus a threshold greater than 70 dB SPL can be considered high It is a clear sign of abnormality when no responses are detectable at levels as high as 110 or 120 dB The latency of the response peak is measured directly from the horizontal time scale of the oscilloscope and can be compared with normative data available for each click level (Montandon et al, 1975*b*) Since latency depends upon stimulus level one effect of a conductive defect is to produce a response that is apparently delayed in latency for a specific click level It should be remembered that responses of auditory nerve fibres tuned to low frequencies are not well represented in the N_1 component (Kiang et al, 1965) Thus the absence of a recordable N_1 in ear canal responses can still be consistent with residual hearing of low frequencies (below 1–2 kHz) Total time involved in the procedure including preparation of the subject setting up of the equipment, actual testing and evaluation of the results takes an average of ½ hour for each ear and usually does not exceed one hour for each subject

RESULTS

Situations where the clinical recording of auditory nerve potentials was found to be useful in determining the further course of action are best illustrated by presenting specific cases Each figure will contain the following data for an individual case

(1) a brief summary of all information available prior to the testing

(2) displays of the recorded averaged responses for selected click levels,

(3) the author's interpretation of the record

Fig 1 No 122 (1) *Clinical history* This child aged 1 year 2 months does not react to sounds He is physically and mentally retarded The referring pediatric neurologist wanted to know whether the ear is also involved in order to choose the most appropriate type of rehabilitation The otoscopic examination is normal There was no reaction to the noise of a "Barany Box" and the Moro-Test was inconclusive

(2) *Averaged Responses*

(3) *Interpretation* The recording made from the right ear shows normal auditory nerve responses The "threshold" is no greater than 70 dB SPL The response latency is within the normal range (dark line below the response peaks) The peripheral auditory system up to and including the auditory nerve is not drastically impaired The hearing disorder is therefore probably of central origin with minimal involvement of the ear No aural rehabilitation was recommended

ings and recommendations made for further management of the case, and

(4) subsequent results, when available

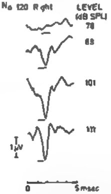
Fig 1 shows data for a child that did not react to sounds The association of severe mental and physical retardation rendered it plausible that the disorder involved the central nervous system It was important however to know if rehabilitation attempts should be supported by acoustic amplification The recording of a normal response from the auditory nerve indicated that the ear was likely to be reasonably intact Thus a hearing aid might not necessarily help in rehabilitation training

Fig 2 shows data for an adult comatose patient who did not respond to speech and sounds as he was emerging from coma Because he had had bilateral temporal bone fractures, extensive damage to the ears was suspected It seemed important to know if the ears were functional in order to determine how rehabilitation should be initiated The recorded ear canal responses indicated that the major damage was probably central, although some conductive loss was also indicated

Fig 3 shows data for a young adult with bilateral hearing loss that was suspected to be of psychogenic origin Normal auditory nerve responses supported the clinical impression, and the patient was sent for psychiatric evaluation, the results of which confirmed the diagnosis of psychiatric problems In contrast, Fig 4 shows data for a young girl who was also

Fig 2 No 120 (1) *Clinical history* This 35 year-old man suffered bilateral temporal bone fractures and brain laceration in a traffic accident As he emerged from coma he did not react to sounds The referring neurosurgeon wanted to know whether the disorder was of central or peripheral origin in order to choose an appropriate type of rehabilitation

(2) *Averaged responses*

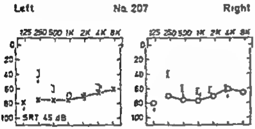


(3) *Interpretation* Averaged responses recorded from the right ear canal show auditory nerve responses at approximately 90 dB SPL and above The "threshold" of response is abnormally high but the deficit is only partial and therefore the lack of reaction to sounds is probably due to a disorder localized more centrally than the auditory nerve A marked delay in response latency suggests that the peripheral deficit is of conductive origin No otological treatment or aural rehabilitation is recommended at this time Suggested reexamination after more recovery of central functions

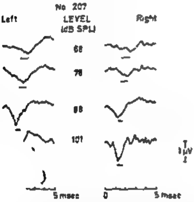
(4) *Follow up* One year later the patient was able to understand conversational speech and to follow orders without amplification or lip-reading

Fig 3 No 207 (1) *Clinical history* This 19 year-old office clerk complained of a disabling hearing loss of gradual onset over the past 8 months He was referred by an otolaryngologist who had treated him unsuccessfully with vasodilators for 2 months He asked if a hearing aid could be of help The otoscopic examination was normal but acuity for speech discrimination appeared to fluctuate The audiogram showed a bilateral deficit of approximately 70 dB at 1 k and of only 45 dB for SRT The discrepancy between thresholds for tones and speech suggested that there might be psychogenic factors However it was felt that an attempt to confirm this clinical impression should be made by recording nerve responses before sending the patient to a psychiatrist

Audiogram



(2) *Averaged responses*



(3) *Interpretation* The recordings made from both ears show normal auditory nerve responses with a threshold no higher than 70 dB SPL The peripheral auditory mechanism is probably intact and the disorder must involve levels higher than the auditory nerve Neither otological treatment nor aural rehabilitation are indicated

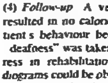
(4) *Follow-up* The patient was referred to the psychiatry ward with a tentative diagnosis of psychogenic deafness The psychiatrist found indications of a psychotic personality with paranoid tendencies complicated by dyslexia

suspected to have psychogenic hearing loss and had been treated as a malingerer by the hospital staff The absence of a recordable neural response indicated a severe deficit of the inner ear or nerve The ear canal recordings proved to be a strong psychological support for the patient and the follow-up showed good results with aural rehabilitation While a correct diagnosis might eventually have been made by other means such as more complete audiological and vestibular testing, none of these methods were as direct as recording the activity of the nerve

Fig 5 shows data for a child who was uncooperative in routine audiometric tests A history of several languages spoken in the home and a complex family situation generated suspicion that the disorder might be of other than organic origin The absence of *neural responses in the ear canal recordings* showed that there were definite signs of malfunction of the peripheral auditory system Once this information was available, the child was referred to an aural rehabilitation centre in her native country where the strain of using several languages would not impose an additional burden on an already defective system

Fig 4 No 151 (1) *Clinical history* This 14-year-old girl recovering from an attack of meningococcal meningitis was referred by the pediatric clinic with a tentative diagnosis of meningococcal labyrinthitis The patient insisted that she could not hear behaved wildly and was very uncooperative at audiometric and otoscopic examination The ward nurses the pediatrician and the examining otolaryngologist all suspected that the patient was either malingering or had psychogenic deafness and a confirmation was sought in nerve recordings

(2) *Averaged responses*

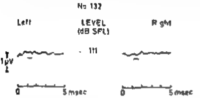


(3) *Interpretation* There is no detectable response even at 120 dB SPL *Thus there proves to be a clear indication of* abnormal ears or nerves and the suspicion of psychogenic deafness is contradicted

(4) *Follow-up* A vestibular test was performed which resulted in no caloric responses on either side The patient s behaviour became markedly improved once her "deafness" was taken seriously and she made rapid progress in rehabilitation (lip reading) Once reliable audiograms could be obtained they showed residual hearing only for very low frequencies on both sides

Fig 5 No 132 (1) *Clinical history* This 4-year old girl from a foreign language family was referred by a pediatrician for hearing evaluation In spite of normal physical development she had not developed linguistic skills and seemed to be mentally disturbed The child was very uncooperative during examination, and the tympanic membrane could not be visualized properly No reliable audiograms could be obtained It was decided to perform the otoscopic examination using general anesthesia (Ketamin) and take advantage of the situation to record from the ear canal while the child was anesthetized

(2) *Averaged responses*



(3) *Interpretation* There is no response at 110 dB SPL The peripheral auditory mechanism is severely impaired on both sides

(4) *Follow up* The conclusion was supported by recording scalp potentials (E R A) which showed some responses only at 90 dB HL in the lower frequency range (250–500 Hz) on both sides It was recommended that this child be sent to a school for the deaf in her own country

Fig 6 shows data for one of two brothers for whom screening audiometrical findings indicated a 40 dB deficit This result seemed to be in conflict with the conduct of the children, who were normally behaved There was some suspicion that the audiometric findings might be due to factors other than organic but in view of the otherwise normal behaviour of the children, more data was desired before one could defend aural rehabilitation based on the use of hearing aids The elevated thresholds of ear canal responses noted in the tests were consistent with the audiometric findings and a concerted effort was made to teach the boys to use hearing aids before they had to enter a more varied environment in which lip reading might not be so reliable

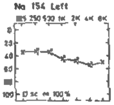
Fig 7 shows data for a mentally retarded girl who had been given a hearing aid on the basis of somewhat ambiguous audiometric data The recordings of normal auditory nerve responses supported the impression that in her case the hearing aid may have been unnecessary and indeed may have added a complicating factor in her rehabilitation

Fig 8 shows data for a young child in whom the ear canal recordings indicated the presence of a conductive loss and led to the discovery of thick middle ear exudates that apparently were not obvious by otoscopic examination

Fig 6 No 154 (1) *Clinical history* This 8 year old boy repeatedly failed the school hearing tests He had been fitted with a hearing aid but refused to wear it and seemed actually to be doing well scholastically without it However he was due to change from a school environment with a single teacher to one with many teachers and it was felt that some attempt sho [illegible]

responses was therefore performed on the better side

Audiogram

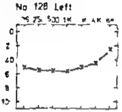


(2) *Averaged responses*

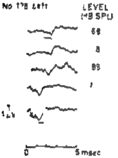
(3) *Interpretation* The recording made from the left ear shows auditory nerve potentials only at high levels suggesting a disorder of peripheral origin consistent with the audiologic findings The latencies are normal Thus a defect of conductive origin is improbable The defect probably involves the cochlea or the nerve A hearing aid was recommended

Fig 7 No 128 (1) *Clinical history* This 8 year old mentally retarded girl had been fitted elsewhere with a powerful hearing aid and now refused to use it She was referred for reevaluation of her hearing problem The otoscopic examination was normal The audiogram showed a bilateral hearing loss of approximately 50 dB at 1 kHz on both sides However her responses were unreliable and a bone conduction audiogram was not obtainable

Audiogram



(2) *Averaged response*



(3) *Interpretation* The recordings made from the left ear show normal auditory nerve responses with a threshold no higher than 70 dB SPL There does not appear to be a drastic impairment of the ear It was recommended that the child should not be forced to use the hearing aid

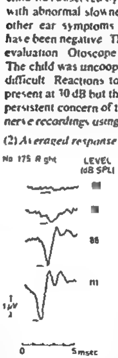
A breakdown of 91 cases that were tested for diagnostic purposes reveals that 4 were infants below the age of 1 year 17 were children aged 1–5 years 18 were children aged 6–15 years 52 were adults The cases involved the following conditions coma 5 mental retardation 8 suspected psychogenic hearing loss 19 suspected central nervous system involvement 5 fluctuating hearing loss of uncertain origin 7 In 47 cases the diagnosis had already been made and confirmation was sought

DISCUSSION

In dealing with a hearing disorder the otologist is interested first in whether he should treat the patient himself or if he should refer him to other specialists It is important for him to evaluate the extent to which the various parts of the ear or the brain are involved In most cases, the patient s history the otological examination, the audiogram and radiological data will already have provided sufficient information to recommend a specific course of action The cases presented here, however are examples where proper management was still in question In some instances recordings from the ear canal were helpful because audiometric data were difficult to obtain (such as the very young or mentally ill, or comatose patients) In others the audiometric and otologic data were insufficient to decide whether

Fig 8 No 175 (1) *Clinical history* This 17 month-old child was observed by his parents and pediatrician to react with abnormal slowness to speech He did not have any other ear symptoms and repeated ENT examinations have been negative The case was now referred for further evaluation Otoscope findings were again insignificant The child was uncooperative and audiometric testing was difficult Reactions to tones and speech appeared to be present at 30 dB but the test was unreliable In view of the persistent concern of the parents it was decided to obtain nerve recordings using Ketamin as a general anesthetic

(2) *Averaged response*



(3) *Interpretation* The ear canal recordings reveal auditory nerve responses for clicks higher than approximately 80 dB SPL However no responses are seen at 70 dB SPL which are usually seen for normal subjects Moreover when the responses were recordable they appeared to have abnormally long latencies suggesting that there may be conductive loss involved Paracentesis was immediately performed on both sides A thick middle ear exudate was found in both ears and a diagnosis of Glue Ears was made

(4) *Follow up* Following drainage the behaviour of the child changed drastically for the better

the defects involved the system up to and including the auditory nerve or more central to the nerve Many cases are seen initially by pediatricians and neurologists who will refer them for testing once they are aware that a useful procedure exists to differentiate ear and brain pathologies In an ordinary otologic practice, cases that require auditory nerve recordings are probably not common

The economic factors in making ear canal recordings cannot be accurately assessed at this time and are constantly changing In the present instance, the apparatus purchased in 1974 cost less than $6000 The canal electrodes are fragile and need to be replaced after use in about 6 ears and cost approximately $3 apiece Each recording session takes approximately ½ hour per ear, and the cost would naturally depend upon who does the recordings In the author's opinion, this relatively modest financial investment is certainly justified in larger medical centres such as a university hospital and may not be out of reach even for individual otologists with a strong interest in the subject

A practical question arises as to who should perform the test otologists neurologists or audiologists? The preparation of the ear canal and the placement of the recording electrode on the canal wall presents no difficulty for an otologist trained in using binocular operating microscopes and those without this training should gain experience in examining the ear canal and tympanic membrane before doing the tests However the training of the audiologist in making careful measurements of behavioral responses to sound is clearly a major asset The interest of the neurologist in further study once the tests indicate central involvement must also be recognized For the moment it would be wise if all specialties were to particip[illegible] and cooper[illegible] in the development of the[illegible] new diagn[illegible]stic methods

ACKNOWLEDGEMENT

The [illegible]uthor wishe[illegible] [illegible] h[illegible]k D [illegible] [illegible] Kiang in Boston USA for his help[illegible] [illegible] [illegible]omments

R T Engel of the Audiology Unit performed most audiograms and other psycho-acoustic tests Technical help was rendered by T Grajew F John G S Roberts H Kappeyne and R Beuchat

RÉSUMÉ

La preuve est faite que l'otologiste peut enregistrer les potentiels du nerf auditif en pratique courante L utilite de tels enregistrements s impose lorsque pour le choix d un traitement il est indispensable de savoir si le trouble est d origine centrale ou périphérique Ils peuvent aussi se montrer fort utiles dans certains cas ou il est difficile voire impossible, d obtenir un audiogramme valable En regle genérale l enregistrement des potentiels du nerf auditif combine avec les examens habituels d audiologie d'otologie et de neurologie permet d'obtenir une image plus precise de l état du malade examine

ZUSAMMENFASSUNG

Die vorgestellte Methode zur Aufzeichnung des Gehornervenpotentials hat sich in der ohrenarztlichen Praxis als Routineuntersuchungsverfahren bewahrt Ausschlaggebend ist das Verfahren im Hinblick auf die einzuhaltende Therapie bei der Unterscheidung von peripheren und zentralen Horstorungen Ein weiteres Anwendungsgebiet ergibt sich aus all den Fallen wo ein zuverlassiges Audio [illegible]

nervenpotentials eine genauere audiologische und otoneurologische Diagnose ermoglicht

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DISCUSSION

Mr *Burian* I should like to ask about your personal experience with the ear canal recording in cases of profound hearing loss We were using a similar technique some years ago but in cases of severe hearing loss it was very difficult or mostly impossible to detect responses [illegible] could be detected clearly with promontory electrodes This was the main reason why we changed our [illegible]ding technique to using promontory electrodes

[illegible]E *Bordley* Can your technique be employed to obtain information on pure tones? A click gives too little information when we are studying a small child and can not be of much help in planning the future training

M Portmann Je suis d accord avec vous qu il est préferable de mettre l electrode dans le conduit plutot que dans l oreille moyenne mais a la condition que les *résultats soient identiques ou ils ne sont pas aussi bons* et aussi complets

1) le prix de l equipment est le meme 2) la simplicite de la pose de l electrode est identique car la peau du conduit est très sensible 3) il faut une anesthesie générale pour la pluspart des petits enfants peut-etre meme plus profonde car l enregistrement est moins bon 4) quelle est la fiabilite au seuil subjectif du sujet? Elle est moins bonne et n atteint pas le voisinage de 100% comme avec la technique de Aran 5) enfin l ECOG est plus qu une recherche de seuil c est aussi l etude de la forme de la reponse en relation avec tel ou tel type de desordre neuro-sensoriel La technique du promontoire est meilleure pour cette recherche

P B Montandon (Reply) to Mr *Burian* We usually do not have responses in cases with profound hearing losses especially if there is no residual function in the higher frequency range

We are indeed not so much interested in exact measurements of residual function but rather in knowing whether the loss of hearing is of peripheral origin or if it is caused by a problem central to the auditory nerve

To Mr *Bordley* It is possible to use pure tones *The recording conditions may however require more sophisticated* laboratory conditions to be reliable In our limited experience we found that information that were most helpful in deciding upon further course of action could be obtained with click responses Pure tone threshold measurements are generally requested for treatment that will require a behavioral audiogram In the present instance we did not intend to produce objective audiometry

To Mr *Portmann* We have compared the responses of the auditory nerve recorded with transtympanic and canal electrodes in animals (P [illegible] Montandon et al 1975) and in humans The amplitude is smaller in the ear canal but it is consistently present and recordable provided the skin resistance is properly taken care of Recordings from the promontory or the round window have a more favorable signal to noise ratio Under these circumstances a wider variety of controlled acoustic stimuli may be used and the behavior of amplitudes latencies and waveforms of various response components can be better analysed in promontory recordings

Our procedure does not require anesthesia in babies that are wrapped up in a towel and held tightly Light sedation may be satisfactory to clear the ear canal in children aged 1 to 5 years The placement of a canal electrode does usually not elicit pain with a trained hand The electrode can remain in the canal for many hours without discomfort Threshold responses may be consistent under controlled laboratory conditions only The type of information we are usually looking for require merely approximate threshold measurements

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SOUND LOCALIZATION WITH PHASE AUDIOMETRY

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Abstract A 500 Hz pure tone is presented binaurally with earphones The tone is adjusted to give a midline impression at comfortable loudness level An electronic variable time delay line unit and a generator for randomizing the shift of the phase lag of the signal to the right or to the left ear respectively are used By shortening the time delay the threshold of the recognition of the phase difference is reached At every test sequence the tone is first presented in the center of the head without delay and then with delay The patient runs the test by indicating in which ear the tone is heard The phase difference is thus gradually reduced from 500 μsec down to a threshold value of about 48 μsec equal on right and left ears in normal hearing subjects

The results of phase audiometry on subjects with normal hearing and with different types of hearing losses are in good agreement with the results of sound localization tests in free field Phase audiometry seems to be of especial value in diagnosing retrocochlear lesions

Directional hearing in the horizontal plane de pends largely upon the acoustic differences between the stimulation of the right and the left ear These interaural differences can be divided into time, phase and intensity differences This important auditory function has been studied in a great number of experiments (Wightman & Firestone 1930 Bekesy, 1930, Jongkees & Groen, 1946, Matzker, 1958, Nordlund 1963, Nordlund & Lidén 1963) The directional hearing in the low frequency range is based on the ability of the individual to detect interaural phase and time differences (Zwislocki & Feldman 1956) The interaural intensity difference is of greatest importance for tones above 1400 Hz However time difference becomes important in directional hearing for clicks white noise or other complex sounds (Christian & Roser, 1957) It has also been shown that directional hearing is better for low frequency pure tones and low pass filtered broad band noise than for high frequency pure tones (Nordlund, 1963)

It is generally agreed upon that the medial accessory nucleus of the olivary complex in the brain stem is the site at which afferent impulses from the two ears first come together Since sound localization is based on time and intensity differences between the ears disturbances of the conduction time of the neural impulses in one of the auditory nerves should register in this measurement Thus lesions of the VIII nerve or the brain stem will render the centre unable to carry out its function properly The fusion of the signal coming from the periphery will not take place in the normal way and will give rise to disturbances in sound localization

Different types of hearing deficits can influence directional hearing Middle ear lesions often impair the directional hearing Lesions of the auditory nerve or the pontile region of the brain give noticeably abnormal directional hearing whereas patients with temporal lobe lesions may have an entirely normal directional hearing Although cochlear lesions can produce slight impairment in directional hearing normal vestibular function is not necessary for the test (Nordlund 1963)

Several methods have been developed for the investigation of directional hearing (Jong

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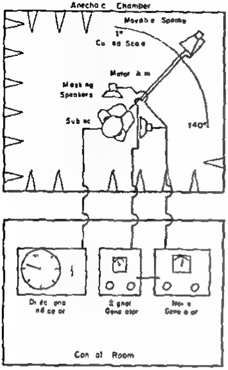


Fig 1 Block diagram of the free field localization test (Nordlund 1963)

kees & Groen 1946 Matzker & Welker, 1959 Nordlund 1963 1964) although two basic tests are presently being used free field tests (directional audiometry) and headphone tests with dichotic presentation (phase audio etry)

The free field method for directional au ometry has been employed routinely in our aboratory for the last 15 years and has proved valuable in the diagnosis of retrocochlear le sions The disadvantage of this test is that it requires an anechoic room The test is performed in the following way A hidden mobile loudspeaker is placed in different posi tions in front of the test subject This is situated behind a recordable scale so that the listerner can respond with the scale number at which he thinks the loudspeaker is located (Fig 1) Clinically the test is now performed with both 500 Hz pure tone and low pass filtered broad band noise as stimuli The sound intensity is set at a comfortable level for the patient but it must be sufficiently intense to be discerned by the more defective ear The patients make 20 judgements for the direction of the tone and 10 determinations for the noise The results of the tests are then analysed with regard to the mean of the differ ences and the standard deviation of the errors of the judgements

In order to attempt to move directional au diometry into regular sound treated rooms we investigated and reported the merits of utiliz ing sound stimulus with variable difference of time or phase between the ears (Nilsson et al 1973) A dichotic presentation of sound over headphones was used in which the equipment was manually operated

The sensation of sound takes the form of an imaginary sound source moving in the head of the listener depending on the interaural time differences (Δt) produced by a delay line Normal listeners start to lateralize this dicho tic sound presentation as soon as a time differ ence of about 20–30 μsec is reached A similar method was used earlier by Matzker (1958 1967) and Matzker & Welker (1959) Matzker found that the position of the phantom sound was disturbed in patients with brain tumours and concluded that his method of examination made it possible to diagnose central hearing disorders and to draw certain conclusions re garding the site of the disease Groen (1969) using similar equipment stated that Δt discrimination inability was only encountered in acoustic nerve disturbances Unilateral dis orders in the temporal lobe were not found to abolish Δt perception although the minimum Δt values might be increased slightly This is in agreement with the findings of Nordlund (1964) but is in contrast to the opinion of Matzker & Welker (1959)

The manually operated phase audiometer was used on 100 normal hearing subjects be tween 15 and 35 years of age Each ear was tested separately and all tests were performed without complications The results of the mean of the smallest delay time values were 46 3 μsec for the right ear and 45 4 μsec for the left ear The standard deviations were 12 8 and 12 2 μsec respectively Of some interest how

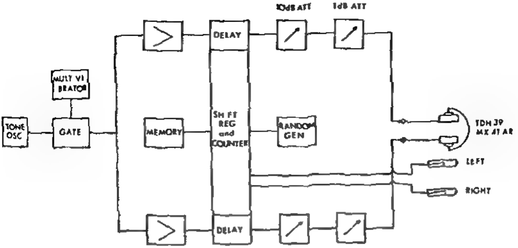


Fig 2 Block diagram of the phase audiometer

ever was the finding that older patients did have difficulty in completing the test It was thus evident that the headphone method had to be modified to facilitate use by the test subject

As a result of these experiences a new automatic phase audiometer was constructed The purpose of this paper is to describe the new method and to report the results of testing normal and some hard-of hearing subjects In addition the merits of phase audiometry in comparison with free field sound localization methods were evaluated

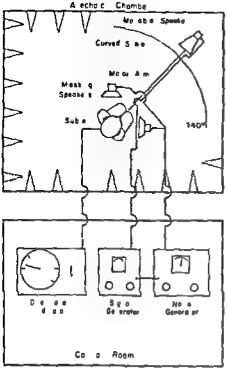
EQUIPMENT

Fig 2 is a block diagram of the phase audiometer The oscillator generates a 500 Hz pure tone which can be presented binaurally either continuously or with pulsed tones (frequency 2½ Hz duration 200 msec rise and decay time 40 msec) over headphones (TDH 39 MX41AR) The maximum out put 110 dB SPL can be adjusted by attenuators in 10-dB and 1-dB steps

The maximum time delay 500 μsec produced by the electronic delay unit gives an experience of complete lateralization of the 500 Hz tone The time delay automatically diminishes in 10 μsec decrements to 200 μsec and thereafter in 5 μsec decrements The generator for randomizing the presentations shifts the phase lag of the signal either to the right or to the left ear At each test sequence the tone used is first presented in the centre of the head without delay and then with delay The patient administers the test himself and is able to record which ear has heard the tone (Fig 3)

PROCEDURE

The 500 Hz tone is presented binaurally with headphones The tone is adjusted to give a midline impression at a comfortable loudness level without delay The test starts by placing the audiometer on automatic position One of the test channels will produce a maximum delay of 500 μsec The patient hears the tone in his opposite ear and records this accordingly The phase lag then disappears immediately The patient again hears the reference tone in the midline position after there occurs a 10 μsec less phase shift presented to one of the ears This phase lag is shifted randomly between ears to minimize preselection by the patient If the subject gives correct responses the phase shift diminishes automatically as previously described A counter samples in correct responses and terminates the test after three errors on each side A flow diagram



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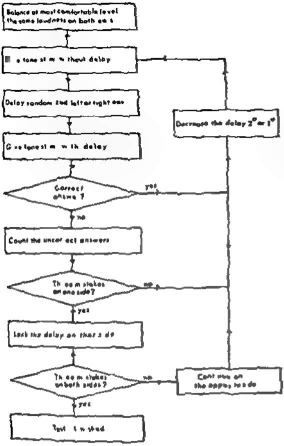

Fig. 4. Flow diagram of the function of the phase audiometer.

tomatic locking of the phase shift after three mistakes also makes the threshold determination easier. In this way, we also avoided the effect of excessive guessing on the final threshold values.

The threshold values on the normal hearing subjects with the automatic phase audiometer correlated well with those values obtained with our manually-operated audiometer. The means of the minimum interaural time delay (ITD) expressed both in microseconds (µsec) and phase degrees on the 60 normal hearing subjects were 47.9 µsec (8.6°) for right ear and 47.8 µsec (8.6°) for left ear (Fig. 5). The corresponding standard deviations were 18.4 µsec (3.3°) and 20.[illegible] µsec (3.6°).

As borderline for possible pathology, the mean of the ITD with three standard deviations was taken, i.e., 105 µsec or 19 phase degrees. All of the 60 normal-hearing subjects studied had their ITD below 95 µsec (17°).

The training effect on the threshold values was analysed on 10 normal hearing subjects. Each subject received six tests with one day's intermission in the middle. As can be seen from Fig. 6, the training effect was found insignificant.

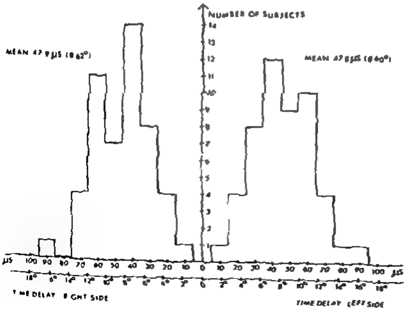

Fig. 5. Distribution of minimum interaural time differences in phase audiometry in 60 normal hearing subjects.

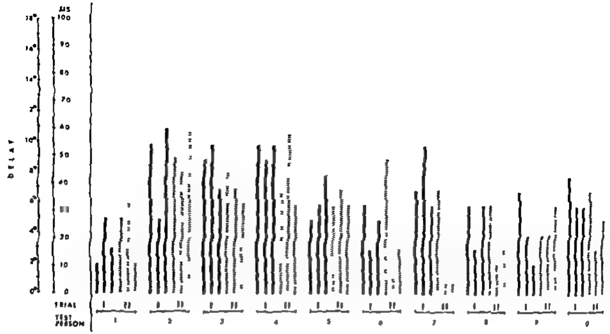

Fig 6 Training effect on 10 normal hearing subjects 6 tests during 2 days

The results of automatic phase audiometry were also compared with the results of free-field directional audiometry (Table I) The mean angle of misjudgment of a 500 Hz signal in a free-field situation was obtained in 100 normal-hearing subjects (20 judgements each), nd found to be 0 6 degrees from true position of the loudspeaker with a 99 9% confidence interval between ±8 degrees Expressed as interaural time difference, these 8 angle degrees correspond to a lag of about 68 μsec The standard deviation for all 2000 judgments was ±2 3 degrees (Nilsson et al , 1973)

A relatively small number of patients with different types of hearing loss have been tested with both phase and directional audiometry simultaneously Otosclerosis gave abnormal thresholds for ITD in 7 of 11 patients (64%) Twelve of 21 (57%) were classified at directional audiometry as abnormal Most patients with cochlear lesions as well as those with intracranial lesions passed the test very well Patients with retrocochlear lesions on the other hand appreciably had abnormal results with both methods

As can be seen from Table I there is good correlation between the two methods studied although there are relatively few patients in whom the two methods have been compared simultaneously For comparison purposes a 500 Hz pure tone was employed as single stimulus in both methods However, it should be pointed out that the clinical evaluation of the results of directional audiometry normally is based on measurements with both 500 Hz pure tone and low pass filtered broad band noise with movable and fixed head respectively If this latter criterion had been applied to the 11 cases with retrocochlear lesions (Table I) there would have been only 2 with normal results at directional audiometry instead of 4 with just 500 Hz as stimulus In addition for those patients with hearing loss studied, the values of testing were compared with those obtained from our normal hearing group

DISCUSSION

Earlier investigations have indicated that the minimum interaural time difference in dichotic presentations which produces an impression of deviation from the midline is approximately

Table I *Results of phase audiometry and free field directional audiometry in normal and hard of hearing subjects Stimulus 500 Hz tone*

	Phase audiometry (min interaural time difference (ITD) in microseconds)						Directional audiometry (angle of misjudgement in degrees)			
	Right ear			Left ear						
Type	n	mean	S D	mean	S D	Remarks	*n*	mean	S D	Remarks
Normal	60	47 9	18 4	47 8	20 0	Normal borderline 105	100	0 6	2 3	Normal limits mean±8 S D 12 from Nilsson et al 1973
Otosclerosis	11	189	144	183	144	7 of 11 had abnormal ITD	21	3 0	11 5	12 of 21 abnormal from Nordlund 1964
Cochlear	16	[illegible]	25	82	24	1 of 16 had abnormal ITD	22	−1 1	6 1	1 of 22 abnormal from Nordlund 1964
Retrocochlear	13	374	123	394	118	12 of 13 had abnormal ITD	11	1 5	12 7	7 of 11 abnormal
Intracranial	4	58	33	[illegible]	33	0 of 4 had abnormal ITD	4	5 5	5 8	1 of 4 abnormal

20 µsec for young normal hearing subjects (Bekesy 1930) In a free field situation an interaural time difference of 20 µsec will correspond to a minimum audible angle difference of about 2 3 degrees The thresholds of interaural time delay determined with the phase audiometer on normal hearing young subjects approach this value However since the test automatically terminates after three misjudgements for each ear our threshold values are somewhat higher

In the free field sound localization test the minimum detectable angle difference is based on time and/or intensity differences between the ears depending on the frequency of the stimulus By turning the head toward the sound source the interaural differences are equalized In this way we sharpen our ability to localize sounds This advantage is of course lost in phase audiometry From the physical point of view phase audiometry could be compared to free-field localization with fixed head

The great disadvantage with phase audiometry is that the subject localizes the sound source in his head or in its close proximity This implies as Nordlund (1963) has already pointed out, that the same degree of accuracy may not be obtained as in the more physiological situation, i e , when sound is presented in free field study Moreover it can also be questioned whether the same response is being investigated since in one situation the sound source is located in the head whereas in the other instance the sound is located in the room Obviously, further study is indicated

From our clinical experiences we can conclude the following (i) phase audiometry can be performed in a regular sound treated room, (ii) the interaural time difference of the barely noticeable phase displacement is longer than for directional audiometry, (iii) phase audiometry is a useful clinical test and provides a means of replacement for directional audiometry in testing patients for possible retrocochlear lesions

RESUME

Un son pur emis sur une frequence de 500 Hz est transmis au sujet binauralement au moyen d une paire d'écouteurs Chaque écouteur dispose d un reglage de volume indépendant permettant de modifier l'intensite

sonore pour chaque oreille séparément jusqu'à ce que le sujet ait l'impression de percevoir le son au milieu de la tête

Un déphaseur – dispositif électronique destiné à provoquer un retardement un décalage dans le temps – et un générateur de panmixie soit de croisement au hasard se chargent de faire passer le son et partant la perception auditive d'une oreille à l'autre dans un ordre purement dicté par le hasard

Chaque série de tests se déroule de la même manière émission d'un son perçu au milieu de la tête d'abord sans retardement puis avec déphasages répartis au hasard

Étant donné que le sujet signale lui même à chaque occasion de quel côté il perçoit le son émis l'examen est entièrement dirigé par lui Le retardement qui au départ est de l'ordre de 500 microsecondes est ramené successivement jusqu'à environ 48 microsec soit jusqu'au seuil d'excitabilité des individus ayant une ouïe normale

Les tests effectués au moyen d'audiomètres à déphasage automatique aussi bien sur des sujets ayant une ouïe normale que sur différents groupes de sujets atteints de surdité concordent largement quant aux résultats obtenus avec les tests portant sur la faculté de localisation des sons en champ libre

La méthode analysée ci dessus peut rendre de précieux services en particulier lors du diagnostic des lésions rétrocochléennes

ZUSAMMENFASSUNG

Ein Ton von 500 Hz wird dem Probanden binaural über Kopfhörer präsentiert Die Tonstärke wird mittels zweier Lautstärkeregler zu einer angenehmen Lautstärke reguliert so daß der Ton mitten im Kopf gehört wird Eine elektronische Zeitverzögerungskette und ein Generator für Randomisierung von den Phasenverzögerungen zwischen den Ohren werden benutzt um bei sinkender Zeitdifferenz Rechts oder Linksempfindungen in einen Mitteneindruck übergehen zu lassen Jeder Testabschnitt beginnt mit einer Präsentation des Tones mitten im Kopf ohne Verzögerung worauf eine Präsentation mit Verzögerung folgt Der Patient lenkt die Untersuchung gänzlich selbst indem er angibt auf welcher Seite er den Ton hört Bei Normalhörenden nimmt hierdurch die Verzögerung gleichmäßig an beiden Ohren von 500 Mikrosekunden stufenweise zu einem Schwellenwert von ungefär 48 Mikrosekunden ab

Bei Untersuchungen von Normalhörenden und verschiedener Gruppen Schwerhöriger hat die Phasenaudiometrie gute Übereinstimmung mit der Richtungsgehörprüfung im freien Feld gezeigt Besonders wertvoll scheint die Methode beim Diagnostizieren der retrocochleären Schäden zu sein

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DISCUSSION

R Hinchcliffe Just a short commentary At a time when many are saying that Bekesy techniques are finished it is refreshing for you to demonstrate the value of this technique applied to something else than the measurement of the threshold of hearing My colleague Robert Danper has recently been using Bekesy techniques for investigating some parameters of binaural hearing He has presented some of his result at the Second British Academy Conference in Audiology

J Tonndorf I was struck by the discrepancy in results between free field and earphone testing in patients with central disorders I see two explanations for this phenomenon 1) that your sound proof room was not really sound proofed 2) that your patients could handle changes in ΔT i e in one parameter at a given time when being tested under earphones In a free field situation there are always simultaneous changes both in ΔT and in ΔI Apparently then your patients were unable to handle such a complex situation both changes occurring in two parameters simultaneously This finding if confirmed could be of diagnostic value

Mr *Schmidt* In directional hearing time (phase)-differences play the major role for frequencies up to some 1200 Hz I wonder if you used frequencies close to this 1200 Hz for instance 1000 Hz beside the stimulus frequency of 800 Hz that you mentioned Particularly in retrocochlear lesions 1000 Hz might be more sensitive than the 500 Hz stimulus

P M Stell You have analysed your results with standard normal (parametric) methods but the histograms of the results appeared to show that they were not normally distributed Did you carry out any statistical tests to ascertain if it was justifiable to use standard normal (parametric) methods?

G Liden (Reply) to Mr *Hinchcliffe* Thank you for your interesting comment We have not used the Bekesy audiometer for this purpose

To Mr *Tonndorf* Our sound proof room is anechoic from 175 Hz up I agree that the free field method seems to be the most sensitive test probably because the patient has to handle simultaneous changes both in Δt and ΔI Because of the trading function between Δt and ΔI there are practical difficulties in phase audiometry to take advantage of both factors

To Mr *Schmidt* We have only used 500 Hz 2000 Hz 4000 Hz and low frequency broad band noise

To Mr *Stell* No we did not

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UPTAKE AND RELEASE OF ALCOHOL BY HOMOGRAFT TISSUES IN TYMPANOPLASTY

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Abstract Alcohol is known to produce severe cochlear damage and it is possible that deafness as a complication of tympanoplasty can be caused by alcohol which remains in homograft materials after inadequate washing An investigation was made in which the quantity of alcohol taken up by ossicles cartilage temporalis fascia dura and ear-drum was estimated The rate of release of alcohol from such materials into saline during washing was also measured The amount absorbed was found to depend upon the nature of the material its weight and its surface area The rate of release of alcohol from these materials differed significantly for each material

High frequency hearing loss or even sub total sensori neural deafness after tympanoplasty is as rare as generally supposed and accord to data collected from colleagues it is complication which most otologists have known It can occur even after a straight-forward myringoplasty Discounting such obvious errors of technique, such as accidental stimulation of the intact ossicular chain by the rotating drill or accidental subluxation of the stapes footplate a number of patients remain in whom the cause is unknown Following a case in which severe hearing loss occurred in a patient in whom an alcohol preserved homograft ossicle was placed onto the stapes footplate as part of a tympanoplasty, it was decided to examine the amount of alcohol taken up by various homograft materials and to assess its rate of release during washing In the case mentioned the duration of washing was not known It was obviously thought to be sufficient by the surgeon concerned (B H C) In the absence of any recognisable technical error or other aetiological factor the homograft ossicle became suspect

Some of the results of our experiments have already been published by Thomas & Colman (1975) This paper presents the results of further studies Data are now available relating to various alcohol-preserved homograft materials, namely ossicles (malleus and incus), cartilage, tympanic membrane, dura and temporalis fascia Homograft tissues preserved in this way continue to be used frequently by otologists as a suitable and satisfactory source of tissue for reconstructive purposes The ototoxic effect of alcohol and other preservatives, however, is nevertheless a factor which should perhaps be borne in mind

Ossicles, dura and tympanic membrane were obtained in the usual way from fresh post-mortem material fascia and septal cartilage were obtained during various ear and nasal operations Five samples of each material were examined Ossicles and cartilage were stored in 90% alcohol for a minimum of three weeks, the other materials were stored in 70% alcohol for the same period as is usual for ordinary clinical purposes For the purposes of the investigation after storage in alcohol each specimen was taken out of its preservative solution, blotted dry and weighed Each specimen was then passed serially through five batches of isotonic saline, being allowed to re-

UPTAKE OF ALCOHOL

OSSICLE	9% OF ITS OWN WEIGHT
CARTILAGE	51% OF ITS OWN WEIGHT
DRUM	25% OF ITS OWN WEIGHT
DURA	21% OF ITS OWN WEIGHT
FASCIA	13% OF ITS OWN WEIGHT

Fig 1 Uptake of alcohol by various tissues expressed as a percentage of the original weight of the tissue

main in each 1 ml batch for half an hour before passing to the next uncontaminated batch After five such washings each homograft was left in another 1 ml of saline for a further 36 hours in order to extract any alcohol which might still remain Washing consisted merely of soaking the specimen in saline without any agitation and between each solution the specimen was rapidly washed in fresh, uncontaminated saline to remove any surface alcohol The alcohol content of each millilitre of normal saline was measured by the gas chromatographic method described by Curry and his colleagues (1966)

It was found that different tissues had different capabilities of absorbing alcohol as indicated in Fig 1 Ossicles took up an average of 9% of their own weight of alcohol compared to 51% taken up by cartilage Drum took up 25% of its own weight, dura 21%, and temporalis fascia 13% of its own weight

The rates for release of alcohol from ossicles and from cartilage are illustrated in Figs 2 and 3 *Ossicular bone after washing for one* and a half hours still contained about a quarter of its original alcohol content By contrast cartilage at one and a half hours had given up all its alcohol Indeed, only a small amount of alcohol remained in cartilage even after washing *for half an hour in spite of the large* quantity present initially in this material Bone presumably because of its more solid structure is much slower to release its contained alcohol

The results with the various soft tissues used for homograft purposes are illustrated in Figs 4, 5 and 6 They show that the rate of release of alcohol from preserved tympanic

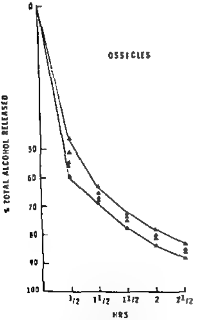

Fig 2 Rate of release of alcohol from ossicles At the start of washing the average alcohol content was 9% of the weight of the specimens examined

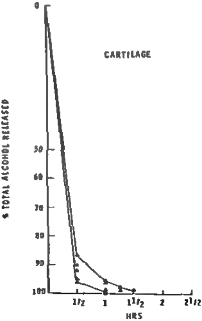

Fig 3 Rate of release of alcohol from cartilage The average initial alcohol content was 51% of the weight of the specimens

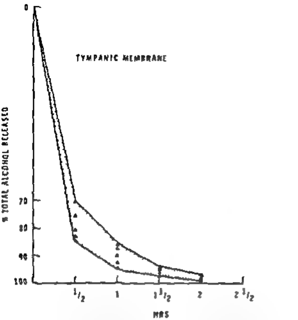

Fig 4 Rate of release of alcohol from tympanic membrane The average initial alcohol content was 25% of the weight of the specimens

membrane was relatively slow, the release from dura was more rapid, release from temporalis fascia was very fast Reference back to Fig 1 shows that these three soft tissues are in fact arranged in the same order in respect of their amount of contained alcohol (in contrast to the situation with ossicles and cartilage)

DISCUSSION

As might be expected, the rate of release of alcohol from these various tissues follows an exponential curve, but the actual rate of release varies with the different tissues In addition, certain tissues have a greater ability to absorb alcohol than do other tissues

Of the tissues used for ossicular reconstruction cartilage was found to be capable of *absorbing a large quantity of alcohol, though* it washes out very rapidly Of the tissues used for drum replacement it was found that tympanic membrane gave up its alcohol slower than the other tissues examined and, furthermore, significantly, was the tissue which during preservation had taken up the greatest amount of alcohol weight for weight It is submitted that there may be potential dangers to the cochlea when preserved tissues are placed on the stapes footplate Ossicles must be washed sufficiently because of the slowness of the

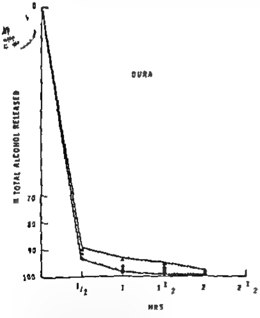

Fig 5 Rate of release of alcohol from dura The average initial alcohol content was 21% of the weight of the specimens

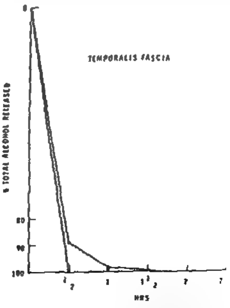

Fig 6 Rate of release of alcohol from temporalis fascia The average initial alcohol content was 13% of the weight of the specimens

release of their alcohol Cartilage must be washed sufficiently because of the large amount of alcohol it contains

Drum replacement tissues probably represent a lesser danger to the cochlea because they are not placed near to the oval window or round window However, tympanic membrane not only washes slowly, but in addition takes up the greatest amount of alcohol By contrast preserved temporalis fascia has poor absorptive powers for alcohol and gives it up very readily

RESUMÉ

L alcool peut endommager serieusement l organe cochleaire et il est possible que la surdite resultante d une complication de tympanoplastie puisse etre causee par l alcool laisse dans les matières homogreffes apres un nettoyage inadequat On a fait des recherches pour estimer la quantite d alcool absorbee par les ossicules le cartilage le fascia temporal la dure mère (dura) et le tympan On a aussie mesure le degre de rejet d alcool de ces matières dans de la saline au cours du nettoyage On a trouve que la quantite d alcool absorbee depend de la nature de la matière de son poids et de sa superficie La quantite d alcool rejetee par ces matières varait de manière significative selon la matière

ZUSAMMENFASSUNG

Es ist bekannt daß Alkohol schwere Cochlearschaden erzeugen kann und es ist moglich daß Taubheit als eine Komplikation von Tympanoplastik verursacht werden kann wenn Alkohol nach ungenugendem Waschen im Homograftmaterial verbleibt Es wurde eine Forschung unternommen in welcher die Menge des Alkohols geschatzt wurde die von Knocheln Knorpeln Temporalis fascia Dura und tympanalen Membranen aufgenommen wurde Es wurde auch die Menge der Alkoholausscheidung von solchen Materalien wahrend des Waschens in Salzlosung gemessen Es wurde gefunden daß die aufgenommene Menge von der Natur des Materiales, seines Gewichtes und der Oberflache abhing Die Schnelligkeit der Alkoholausscheidung von diesen Materalien unterschied sich bedeutend für jedes Material

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DISCUSSION

T Palva 1) Would it not be better to use the term tissuetoxicity instead of ototoxicity to describe the action of alcohol? 2) I should like to suggest washing your grafts in the future in distilled water which I have been using for many years We all know from the histological tissue techniques that if you want to get rid of alcohol you wash with distilled water not with saline I venture to suggest that if you repeat your experiments with distilled water you need much shorter time to remove the alcohol

K Sedlacek Are there any changes in the biological activity of the grafts caused by alcohol? We observed that the cellular elements of the skin preserved with alcohol did not grow in tissue culture at all or very slowly

O Connor (Reply) to Mr Palva I agree that alcohol has a general tissue toxicity it is ototoxic as well and the experiment was carried out purely in this specific context It may well be safer to wash homografts in water rather than saline we have not studied the rate of release into water we should perhaps use water in view of your comment

To Mr *Sedlacek* Yes implant of autograft tissue always carries some risk of adverse reaction in the cochlea and elsewhere This experiment however was purely confined to a study of alcohol in and out of grafts

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ULTRASTRUCTURAL INVESTIGATIONS ON THE DEGENERATIVE AND REGENERATIVE PROCESSES IN CHORDA TYMPANI IN BELL'S PARALYSIS

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Abstract Our ultrastructural findings in investigated cases of Bell s palsy have demonstrated that the degenerative changes did not proceed at the same speed from the onset of the paralysis—in other words the regeneration changed in accordance with the topography of the nerve Although the endoneural sheath preserved its integrity swellings in the Schwann cells have attracted much attention Furthermore the perineural tissue presented changes which were closely correlated with the degenerative and regenerative processes due to paralysis In addition to these findings although the interval between the onset of paralysis and the operation was the same in all our cases the observation of a more severe degenerative picture of the nerves in the female patients may seem to be an interesting finding but to the lack of an adequate number cases which would enable a statistical evaluation re strongly of the opinion that this subject deserves er study

There is almost total agreement among surgeons concerned with the treatment of facial nerve paralysis, as the origin of this condition is quite clear In Bell s palsy, however, there is still a certain amount of controversy regarding both the attributing to this lack of agreement is the paucity of histopathologic investigations on the facial nerve

As will be recalled, Minkowski (1891) was the first to study the morphology of Bell's palsy in a case where he noted that the nerve was normal in its course from the nucleus to the genicular ganglion, but that the part which lay within the Fallopian canal was very edematous These findings were followed by the similar observations of Dejenne & Theohari (1897), Jongkees (1954) and Miehlke (1959)

On the basis of the above observations, we have used the electron microscope in an attempt to evaluate the structural changes in the chorda tympani in cases of Bell's palsy and thereby to be of some help in this subject

MATERIAL AND METHOD

The entire course of the tympanic chord, removed surgically in cases of Bell's palsy with taste bud defects, was investigated by means of the electron microscope Decompression was achieved clinically by the retroauricular method, the cavum tympani was then reached through a second incision and the chorda tympani removed at the very beginning of the operation Maximum care was taken not to

Table 1 *Subject material*

Patient	Age	Sex	Time of the decompression procedure (months after onset of paralysis)
F B	28	♀	Otosclerosis (Control)
Z O	24	♀	3 months
A K	49	♂	3 months
Y S	20	♀	2½ months
N U	19	♂	3 months
C T	40	♂	4 months
H D	50	♀	3 months

Fig. 1. The otosclerotic case (F. B.) taken as a control. The ultrastructure of the tympanic chord is shown. (*a*) At low magnification a regular myelin structure and basement membrane can be visualized. ×35 000. (*b*) A high resolution picture of the myelin lamellae. ×99 000.

cause the slightest trauma. Furthermore in order to compare the chorda tympani obtained from patients with Bell's palsy with a normal specimen we removed the chorda tympani from an otosclerosis patient on whom we performed a stapedectomy. This chorda was also submitted to electron microscopical investigation. Six patients (3 of them women)

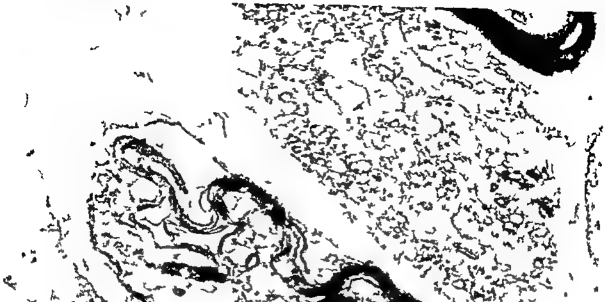

Fig 7 A case of Bell s palsy (Z Ö) where extens ve degenerative f nd ngs are observed A pronounced ir regularity can be noted in the structure of the nerve espec ally n the myel n lamellae The ba ement mem brane and the endoneural sheath howe er ma n ain the r integrity ×14000

were studied 2½–4 months after the onset of Bell s palsy Their ages ranged between 19 and 50 (Table I)

Following our electron microscopy tech nique we sectioned the biopsy specimens at 1 mm in a droplet of isotonic solution The sections were fixed for one hour at +4°C in a solution of Veronal acet te and osm um tetroxide buffered to pH 7 2 The pecimens were then dehydrated in an acetone series and embedded in Vestopal W before ect on ing at 300–500 Å on an LKB ultr t me III Finally these thin sections were sta ned with uranyl acetate and lead citrate for tudy in

Fig 3 A section of the tympanic chord from a 20-year-old female patient (Y S) Mild degenerative changes were observed in the ultrastructure of the nerves ×14000

the Zeiss Em 9 2S and JEOL 100 C electron microscopes

Alternatively 1 μ thick sections were cut from the plastic block prepared for electron microscopy and stained with the Giemsa technique for observation under the light microscope

FINDINGS

Case I (Z O)

Taste bud disturbances were noted at the pre operative examination of this 24 year old fe male patient who underwent decompression 3 months after onset of the paralysis

Electron microscopic findings Besides an

Fig 4 A section taken from another part of the tympanic chord of the same patient (Fig 3) The degenerative changes were more pronounced the myelin lamellae in particular present extreme disorder ×23 800

advanced disorder of the myelin lamellae additional findings were made at the ultra structural level described as hydropic changes in the myelin by Miehlke when studied under the light microscope (Fig 2)

Case 2 (A K)

In this 49 year old male who underwent decompression 3 months after the onset of paralysis taste bud disturbance was also noted at the preoperative examination

Electron microscopic findings A hydropic picture was dominant in the myelin sheath which was degenerated to a lesser extent and changes were observed in certain foci

Case 3 (Y Ş)

A 20 year old female who underwent decompression 2½ months after the onset of

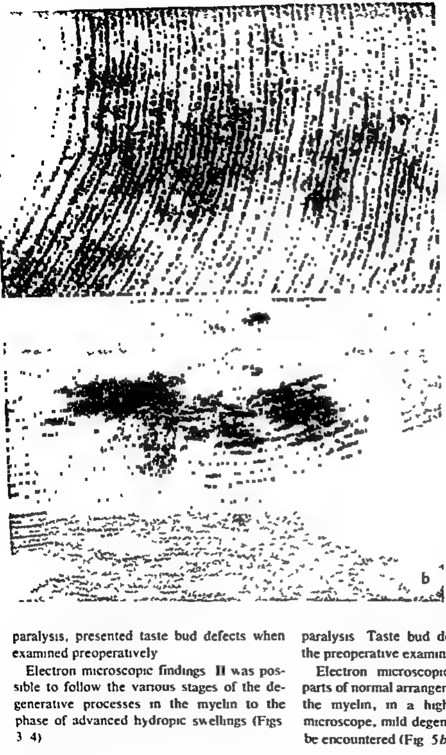

Fig 5 High resolution pictures of the myelin lamellae (*a*) From control case (F B) ×261 000 (*b*) From a case of Bell's palsy (N U) ×225 000

paralysis, presented taste bud defects when examined preoperatively

Electron microscopic findings It was possible to follow the various stages of the degenerative processes in the myelin to the phase of advanced hydropic swellings (Figs 3 4)

Case 4 (N U)

A 19-year-old male patient who underwent decompression 3 months after the onset of paralysis Taste bud defects were found at the preoperative examination

Electron microscopic findings Scattered parts of normal arrangement were observed in the myelin, in a high resolution electron microscope, mild degenerative features could be encountered (Fig 5*b*)

Case 5 (C T)

A 40-year-old male, subjected to decompression 4 months after the onset of paralysis

Fig. 6. The electronmicrogram of a case of Bell's palsy (C. T.), where slight degenerative changes were observed in the tympanic chord. The myelin lamellae are of uniform appearance in most nerves. The basement membrane and the endoneural sheath are of regular structure. ×13 700

Taste bud defects were present in the examination performed during the preoperative period.

Electron microscopic findings. Foci of regeneration were present along with degenerated areas. It can be postulated that the 4 month interval played a part in the establishment of the mentioned regeneration (Fig. 6).

Case 6 (H. D.)

A 50-year-old female patient, subjected to decompression 3 months after the onset of

Fig 7 A section of the tympanic chord from a 50 year-old female patient (H D) Extensive degenerative disorders due to Bell s palsy can be noted ×23800

paralysis Taste bud defects were detected at the preoperative examination

Electron microscopic findings An advanced hydropic degeneration was dominant in the myelin sheath which represented the appearance of a severe condition (Fig 7)

DISCUSSION

As mentioned in the introduction, although surgeons who deal with the treatment of facial nerve paralysis share the same opinions, the fact that the aetiology of Bell's palsy still remains obscure and the presence of differing views concerning its treatment raises a problem for those who are faced with such cases

In this study, undertaken in order to shed further light on this subject, the reason for our investigating these cases 2½ to 4 months after the onset of the condition is that some cases

heal spontaneously without the need of treatment Therefore, patients who are aware of this fact do not always agree to undergo an operation during the early stages Hence we could only follow the changes which developed 2½ months after the onset of the paralysis For this reason we have been somewhat confused in the interpretation of whether the various nerve structures were due to a development where a regeneration had also taken place, or whether this aspect arose from the fact that the degree of degeneration was slight in the observed areas (Figs 3, 4, 6)

In the investigation of the material taken from our cases, the presence of an almost normal aspect besides a progressing regeneration, forces us to think in both ways (Figs, 3, 4)

The very fact that even light microscope observations are very few in the histopathological investigation of the facial nerve in non-lethal cases of Bell s palsy has rendered the establishment of comparisons for the assessment of our ultrastructural findings extremely difficult Moreover, the first observations of Minkowski (1891) and Dejerine & Theohari (1897) who reported the presence of swellings in the Schwann sheath but who could not serve inflammatory changes, were comple ented by the findings of Jongkees in 1954 and Miehlke in 1959 In the reports of the latter authors hydropic changes in the myelin sheath around the axons have been mentioned It is a well known and accepted fact that further detail on this subject cannot be obtained via the use of the light microscope Our ultrastructural findings especially the observations where various degrees of degenerative change are seen in the lamellar formations within the myelin sheaths are rather important

Various degrees of change in the myelin lamellae are very clear in the electron micrograms of our cases (Figs 2–7) *It is obvious* that only the advanced impairment of the myelin lamellae and the vacuole like structures as seen by the mentioned authors who had no choice but to use the light microscope Additionally it is impossible to demonstrate the slight degenerative changes of the myelin lamellae by means of the light microscope It is a fact that these little impairments could only be demonstrated using a high resolution electron microscope, as is seen in Fig *5a–b*

It might be appropriate here to mention an interesting observation in which low magnification electron micrograms prepared from two different cases of Bell s palsy were compared

Despite the fact that only slight impairment of the myelin structures could be observed in one case, we have faced a picture where extremely degenerative myelin structures were dominant in the second case (Figs 2, 6) and the comparison of these two cases is interesting in several respects The case in which a myelin picture close to normal (Fig 6) was observed was that of a male patient who had been operated 4 months after the onset of the disease, the other case where extensive myelin degeneration (Fig 2) was detected, was a female operated upon 3 months after the onset of paralysis

The discussion here can be held in two paths Of course the first thing which will come to mind is that the period of time between the onset of paralysis and the operation will be a positive and favourable factor in the establishment of regeneration Furthermore *a question can be raised* as to whether the fact that these two cases were of different sex could play a part in this dissimilarity

In fact, when two other Bell s palsy patients who had been operated 3 months after the onset of paralysis were investigated it was found that the ultrastructural structures of the nerves were less degenerative in relation to those of the mentioned female (Fig 7)

We also want to draw attention to the fact that there can be a difference in the prognosis *of the other two of our cases* one a woman and the other a man One of these cases a male patient 49 years old (A K) was operated 3 months after the onset of

paralysis and the myelin elements were found to be close to normal, the other, a 50-year-old female patient (H D) was also operated 3 months after the onset of paralysis but clearly presented evidence of grave degeneration of the myelin sheath in the ultrastructural investigation of the nerves, although there was no difference in age between the two patients

When we direct our discussion towards the evaluation of the Schwann cells taken from the nerve material we can add that we have similarly noted the same swellings described by Minkowski (1891) in our electron microscopic investigations

When the perineural tissue was investigated, this tissue followed rather clearly as a structure surrounding the endoneural sheath around the nerves as seen in Fig 6 which represents our case which showed so little degeneration as to imply the presence of extensive regeneration, and it also presents a parallelism with the structure of the nerve section of the tympanic chord (Fig 1*a* *b*) taken from the otosclerotic case which we accepted as normal

In contrast the determination of obvious impairments and swellings (edema) in the perineural tissues of cases where an advanced state of degeneration was observed can prompt one to postulate that Bell s palsy also effects the perineural tissue

RÉSUMÉ

Nos recherches ultrastructurelles sur des cas de paralysie de Bell nous ont démontré que les changements dégénérescents sont moins rapides au début de la maladie En d autres termes la régénération varie selon la topographie du nerf Alors que la couche endoneurale conserve son intégrité le gonflement des cellules de Schwann attire l attention D autre part le tissu périneural présente des modifications en corrélation avec les effets dégénérescents de la paralysie Il nous a paru intéressant de constater que les effets de dégénérescence sont beaucoup plus marquées chez les sujets de sexe feminin Mais le nombre de cas ne permettant pas de réaliser une statistique valable nous avons l intention d en faire l objet d une etude ultérieure

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ZUSAMMENFASSUNG

Die ultrastrukturellen Befunde der untersuchten Bell Paralyse Fälle haben uns gezeigt dass die Geschwindigkeit der degenerativen Prozesse anfangs nicht überall gleich ist anders ausgedruckt, dass die Regeneration von der Topographie des Nerves abhängig ist Im Gegensatz zur völligen Erhaltung der endoneuralen Scheide ziehen die Auschwellungen der Schwannschen Zellen die Aufmerksamkeit auf sich, ausserdem zeigen sie Veränderungen, die den degenerativen Vorgangen entsprechen und sich auf die perineurale Paralyse beziehen Trotz der gleichen Zeitdauer vom Anfang her bis zum chirurgischen Eingriff verlaufen die degenerativen Prozesse in den Nerven bei den Frauen schwerer als bei den Männern Dieser Befund ist neben den anderen auch interessant Aber unser Meinung nach sollen solche Untersuchungen noch weiter durchgeführt werden weil unsere Befunde vom Anzahl her statistisch nicht bewartet werden können

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DISCUSSION

U Fisch Have you seen cellular (inflammatory) reactions in the chorda tympani? Have you correlated the severity of the lesion of the chorda tympani with the amount of degeneration of the motor fiber (as determined by electro-diagnosis)?

L B W Jongkees Have you found any sign of inflammation in the diseased chorda tympani? I ask this since authors (especially in the US) suggest infection as the source of Bell s palsy although a sign of inflammation has never been described in this kind of cases in the microscopic era Did you find viruses in your preparations?

M S Karatas (Reply) to Mr *Fisch* As we have observed small areas it wasn t easy to demonstrate whole chorda tymp ri in our electron micrographs and see the inflammatory reaction But I think it may be possible to demonstrate the signs of inflammation if we use the

light microscope in the following part of our investigation

As for the severity of the lesion, I would like to draw your attention to our morphological findings in the ultrastructural level which I have shown in the slides As demonstrated it is extremely interesting to observe that although there were very well preserved areas, some regions of the chorda tympani showed an advanced state of degeneration in the same case It is rather difficult to say if they were due to a mild case or if there was some regeneration taking place

In relation to the correlation of the severity of the lesion of the chorda tympani with the amount of degeneration of the motor fiber I could only say that the preoperative examination of the patients showed a total denervation in accordance with the electromyographic findings In addition, taste bud disturbances and stapes reflexe defects were noted in the same patients

To Mr *Jongkees* I think my first answer to Mr Fisch s question also is the reply to the first question of Mr Jongkees Answer concerning the viruses is negative We could not find any virus in our electron microscopic preparations

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ENZYMDIAGNOSTIK IM MENSCHLICHEN PAROTISSPEICHEL UND IHRE PROBLEME

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Abstrakt Für die drusenspezifischen Enzyme Amylase, Lysozyme und Kallikrein werden die Aktivitaten von gesunden Kollektiven und ihre Abhangigkeit von der Flußgeschwindigkeit untersucht Dabei ist die Angabe von Normbereichen fur differenzierte Sekretionszustande günstig Im Gegensatz zur Amylaseaktivitat fällt die Aktivitat von Lysozym und Kallikrein mit steigender Flußgeschwindigkeit stark ab Die in der Zeiteinheit sezernierte Aktivitat nimmt zu Ein Vergleich der Enzymaktivität bei Erkrankung der Parotis mit den Normwerten zeigt bei chronischen Prozessen eine signifikante Erniedrigung von Lysozym und Amylaseaktivitat Akute Entzundungen verandern die Amylaseaktivitat gering erhohen die Lysozymaktivitat dagegen signifikant Parotismischtumoren fuhren zu keinen sicheren Anderungen der Enzymaktivitat Die Auftrennung von Amylase in Isoenzyme durch Polyacrylamidgel Elektrophorese und ihre mögliche Bedeutung für das Erkennen pathologischer Prozesse wird diskutiert Uber erste Ergebnisse bei der Auftrennung des Parotislysozyms wird berichtet

Fur die Differentialdiagnostik von Erkrankungen der Speicheldrusen gewinnen Enzymuntersuchungen wachsende Bedeutung Der hohe Grundumsatz und der Enzymreichtum des Parotisgewebes ermöglichen die Untersuchung von Enzymaktivitatsänderungen in den Drusensekreten bei pathologischen Prozessen im Gewebe

Dabei sind diese Enzyme in enger Beziehung zu moglichen Änderungen des Proteinmusters der Druse zu sehen Die Auftrennung mit dem derzeit leistungsfähigsten elektrophoretischen Trennverfahren liefert mehr als 20 unterschiedliche Proteinbanden, deren Zuordnung zu bekannten Proteinen bisher nur in wenigen Fallen moglich ist Neben drusenspezifischen Proteinen treten in unterschiedlicher Menge Proteine aus dem Blut auf Das Proteinmuster der Parotis weist dabei bei einem Normalkollektiv eine betrachtliche Variation auf, deren Ursache genetisch fixierte Muster sein können Individuell dagegen ist das Proteinmuster nach Zahl und Art der getrennten Proteine relativ konstant und zeigt nur quantitative Unterschiede bei verschiedenen Sekretionszustanden (Steiner & Keller, 1968, Smith et al , 1974, Munzel & Eichner, 1974)

Ziel der Proteintrennung im Sekret der Speicheldrusen ist es, genetisch oder funktionell bedingte Ursachen der Variabilitat der Speicheldrusenproteine zu erkennen und sicher unterscheiden zu konnen Voraussetzung hierfur sind standardisierte Sekretgewinnung und reproduzierbare Trennungen (Smith et al , 1974) Bisher ist es möglich, die Proteinveranderungen bei Systemerkrankungen (Sjogren, Aldosteronismus, zystische Fibrose) diagnostisch auszuwerten

Die Untersuchung der Enzyme innerhalb des Proteinmusters wird durch die Moglichkeit spezifischer Reaktionen erleichtert, so daß ihre Zuordnung weniger schwierig ist Dabei verdienen drüsenspezifische Enzyme Amylase, Kallikrein) das Hauptinteresse Untersuchungen befassen sich aber auch mit Enzymen, die nur teilweise in der Parotis synthetisiert werden (Lysozym) oder aus dem Blut stammen (Laktatdehydrogenase – LDH) Die Aussagefähigkeit von Enzymaktivitätsmessungen wird durch die große biologische

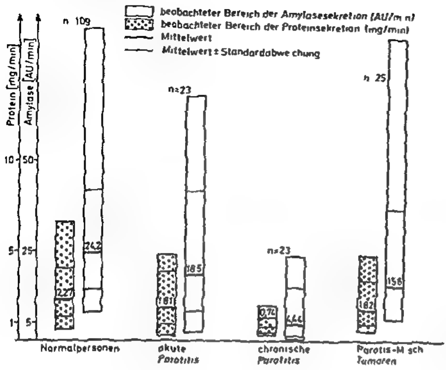

Abb 1 Mittelwerte Standardabweichungen und beobachteter Bereich von Protein und Amylaseaktivität eines *Normalkollektivs* und bei Erkrankung der *Parotis*

Schwankungsbreite und eine starke Abhängigkeit vom Sekretionszustand der Drüse eingeschrankt

In der vorliegenden Arbeit wird über die Aktivitatsmessung von Amylase, Kallikrein und Lysozym berichtet, differenzierte Normeiche unter Berücksichtigung des Sekrenszustandes werden angegeben und die öglichen Aussagen einer weitergehenden Auftrennung in Isoenzyme werden für Amylase und Lysozym diskutiert

METHODE

Die Parotissekretgewinnung erfolgt mit einer modifizierten Curby-Kapsel unter relativen Ruhebedingungen und nach oraler Stimulation mit Vitamin C, wobei die Proben nach Entleeren der Drüse gewonnen werden Die Flußgeschwindigkeit wird mit einem Tropfenzahler gemessen oder nach Auswagen der Probe mit der mittleren Dichte berechnet Die Protein- und Amylasebestimmungen erfolgen nach Skurk et al (1975) Die Lysozym- (Gorin et al , 1971) und Kallikrein-Aktivitatsmessungen (Trautschold, 1970) basieren auf bekannten Methoden und erfolgen mit dem Eppendorf-Gerätesystem (Eppendorf-Gerätebau, Hamburg) Die elektrophoretischen Trennungen erfolgen in Polyacrylamidgel mit einer Universal-Trennkammer (VEB Carl Zeiss, Jena)

ERGEBNISSE UND DISKUSSION

Amylase

Bildungsorte fur α-Amylase (EC 3 2 1 1) in der Parotisdrüse sind die Zellen der Acini und benachbart liegende Kanalzellen (Kraus & Mesteky, 1971) Die elektrophoretische Auftrennung in Isoamylasen und deren unterschiedliche Struktur sind untersucht worden (Keller et al , 1971)

Abb 1 zeigt die Mittelwerte, berechnete Standardabweichungen und den beobachteten Bereich der Amylase- und Proteinsekretion (AU/min , mg/min) bei gesunden Personen und Patienten mit Erkrankungen der Parotisdrüse, die eine log-normale Verteilung zeigen Bei 23 Patienten mit einer akuten Parotisschwellung, verursacht durch Bakterien oder Viren, werden dabei keine signifikanten Un-

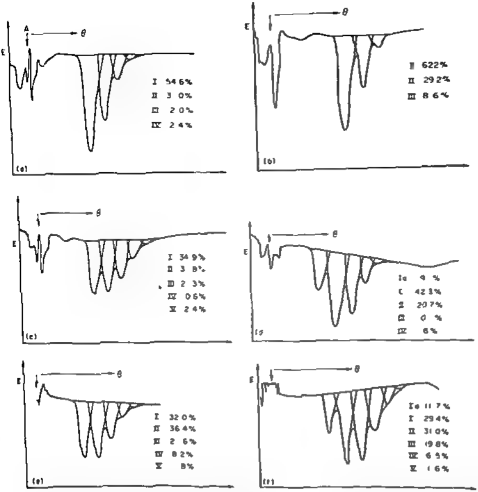

Abb. 2. Extinktionskurven der Amylase-Zymogramme. (*a*) Normalverteilung. (*b*) Normalverteilung bei 4 Personen. (*c*) Probe nach Aufbewahrung (20^d, 4°). (*d*) Genetische Variation mit einer zusätzlichen kathodischen Bande. (*e*) Speichelprobe nach Bestrahlen der Parotis mit 1350 r. (*f*) Gleiche Probe wie (*e*) nach III stündigen Stehen bei 4°. (Aus A. Skurk, D. Fritsche und K. Fendel, 1975, Archs Oral Biol *20*, 429.)

terschiede im Vergleich mit dem Normalkollektiv beobachtet. Signifikant erniedrigt sind Amylase- und Proteinsekretion bei einer Patientengruppe mit chronischen und rezidivierenden Prozessen, die auch eine niedrige Flußgeschwindigkeit haben. Parotis-Misch-Tumoren führen zu keinen signifikanten Änderungen.

Bei chronischen Entzündungen der Parotis nimmt mit steigender Flußgeschwindigkeit der Proteingehalt ab ($r=-0{,}3$), was auf eine gestörte Proteinsynthese schließen läßt.

Die Auftrennung der Amylaseisoenzyme liefert unter den gewählten Bedingungen (Skurk et al., 1975) 4 Fraktionen, deren halbquantitativen Auswertung durch eine nichtlineare Eichkurve erschwert wird. Sowohl Zahl als auch Aktivitätsverhältnis der nachweisbaren Isoenzyme sind konzentrationsabhängig, so daß nur bei strenger Einhaltung der Verdünnung reproduzierbare Werte erhalten werden.

Über 90% aller gesunden Personen besitzen unter diesen Bedingungen ein Isoamylasemuster, in dem die beiden Hauptisoenzyme weniger als 10% (Variationskoeffizient)

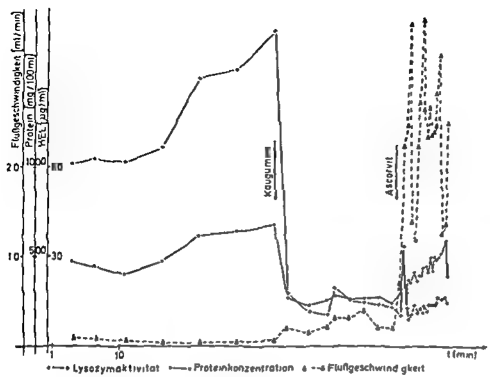

Abb 3 Proteinkonzentration und Lysozymaktivitat im Parotisspeichel in Abhangigkeit von Zeit und Stimulationszustand

schwanken (Abb 2*a*) Das Auftreten zusätzlicher Banden oder von der Normalverteilung abweichende Aktivitaten kann genetische Ursachen haben (Abb 2*d*) Das Verhältnis der Isoenzyme verandert sich bei Aufbewahrung, was moglicherweise auf einen Abbau in niedermolekulare Bruchstucke zuruckzuführen ist (Abb 2*c*) Eine gleichartige Anderung wird auch beobachtet, wenn durch Storung der Sekretion Flußgeschwindigkeit und Amylasesekretion erniedrigt sind, was bei chronischen Prozessen oder nach Bestrahlung der Drüse beobachtet wird Durch Bestrahlung kann es auch in vitro zu Anderungen des Musters kommen (Abb 2*e* u *f*)

Bei Ausschaltung methodischer Fehler gestattet die Auftrennung der Amylaseisoenzyme das Erkennen von funktionellen Storungen, ohne aber entscheiden zu konnen, ob es sich bei verandertem Muster um Synthesestorungen oder lediglich um einen Alterungsprozess in vivo handelt

Lysozym

Lysozym (EC 3 2 1 17) wird in der Parotis von Spezialzellen abgesondert, die sich von der Basalmembran zum Rohrenductus erstrekken und deren Kerne peripher zu den Kernen der Striatazellen liegen Eine zweite Quelle der Lysozymaktivitat sind orale Neutrophile und Monozyten, die in das Speicheldrusengewebe eindringen und zerfallen

Lysozym besitzt bakterizide Aktivitat und ist daruber hinaus in der Lage, durch Bindung an das Sekret – Ig A der Parotis diese Aktivitat auf Ig A zu ubertragen

Die individuelle Abhangigkeit der Lysozymaktivitat von der Flußgeschwindigkeit zeigt Abb 3 Mit Steigerung der Flußgeschwindigkeit fallt die Aktivitat, sie bleibt im Gegensatz zur Proteinkonzentration auch nach Stimulation mit Ascorvit niedrig Die relative Konstanz unter Ruhebedingungen und nach Stimulation ermoglicht die Angabe von sinnvollen Normbereichen bei zwei Sekretions-

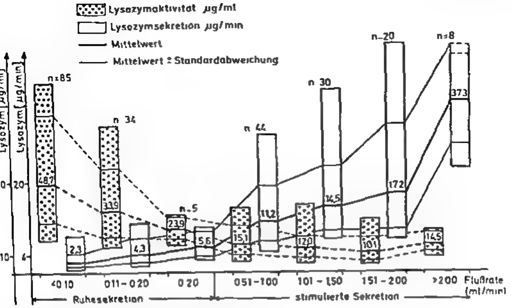

Abb 4 Mittelwerte Standardabweichung und beobachteter Bereich der Lysozymaktivität im Parotisspeichel in Abhängigkeit von der Flußgeschwindigkeit

zuständen mit nur zwei Messungen Die biologische Schwankungsbreite der Aktivitäten vor und nach Stimulation zeigt Abb 4

Die Abhängigkeit der Lysozymaktivität von der Flußgeschwindigkeit macht es erforderlich differenzierte Normbereiche für verschiedene Flußgeschwindigkeiten anzugeben Dadurch werden engere Normbereiche erhalten, die pathologische Abweichungen besser erkennen lassen Die Abbildung enthält die Mittelwerte, die berechneten Standardabweichungen und den beobachteten Bereich der log normalen Aktivitätsverteilungen Durch eine Unterteilung des Normkollektivs werden bei extremen Flußgeschwindigkeiten die Werte wegen geringer Probenzahl unsicher Ein Vergleich der gewählten Bereiche zeigt die stetige Abnahme der Lysozymmittelwerte mit höherer Flußgeschwindigkeit, während die in der Zeiteinheit sezernierte Aktivität ansteigt

Die Abb 5 zeigt Mittelwerte und beobachteten Bereich der Lysozymsekretion vor und nach Stimulation bei Parotiserkrankungen im Vergleich zum Normalkollektiv Bei 12 Patienten mit akuter Parotisschwellung, deren Serumamylasewerte erhöht waren, ist ein Anstieg der Lysozymaktivität zu beobachteten Eine Gruppe mit histologisch gesicherter chronischer Entzündung besitzt signifikant niedrige Lysozymaktivitaten Flußgeschwindigkeit, Proteinkonzentration und Amylaseaktivität sind bei diesem Krankheitsbild gleichfalls niedrig Rezidivierende, eitrige Parotiserkrankungen führen im Mittel zu einem Anstieg der Lysozymsekretion In dieser Gruppe blieben 2 Patienten unberücksichtigt, deren Aktivitaten den beobachteten Normbereich um das 10-fache übersteigen Parotis Misch-Tumoren führen zu keinen signifikanten Lysozymänderungen, wenn das umgebende Drüsengewebe keine Entzündungszeichen zeigt Abb 5 enthält auch den beobachteten Aktivitätsbereich bei 14 Patienten, die wegen chronischer Schleimhaut- und Knocheneiterung, rezidivierenden Cholesteatomen und chronischen Kieferhöhlenprozessen behandelt wurden, deren Parotisdrüsen aber klinisch unauffällig sind Die signifikante Erhöhung der

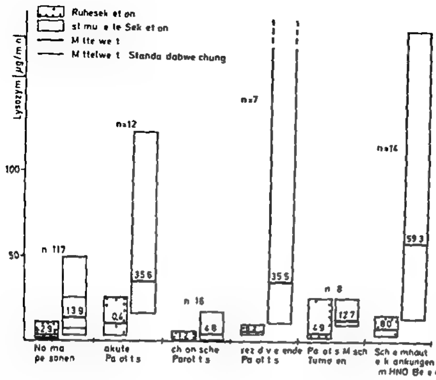

Abb 5 M ttelwerte und beobachteter Bere ch der Lysozymsekret on e nes Normalkollektivs und be Erkrankung der Parotis vor und nach St mulation

Lysozymaktivitat bei normaler Flußgeschwindigkeit und Proteinkonzentration deutet auf eine allgemeine Steigerung der Lysozymsekretion der Parotis bei entzundlichen Prozessen Schleimhaut hin

ıngeklart ist bisher, ob die Erhöhung der sozymaktivitat bei bestimmten pathologischen Prozessen auf eine vermehrte Synthese in den Drusenzellen oder auf steigendes Eindringen von Leukozyten in das Drusengewebe zuruckzuführen ist Dabei steht der Nachweis uber das Vorliegen von Isoenzymen und ihre Trennung noch aus

Abb 6 zeigt die elektrophoretische Trennung im Bereich der basischen Speichelproteine und die Fraktionen mit Lysozymaktivitat Ob es sich bei den 3 Fraktionen um Isoenzyme handelt kann noch nicht entschieden werden

KALLIKREIN

Kallikrein (EC 3 4 4 21) gehort zu den Peptidylpeptid Hydrolasen zeichnet sich aber durch eine hohe Substratspezifitat aus Es spaltet aus dem in der Globulinfraktion vor

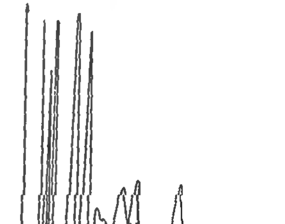

Abb 6 Polyacrylamidgel Elektrophorese von Parotisprotein m t Lokal sat on der Lysozymakt at

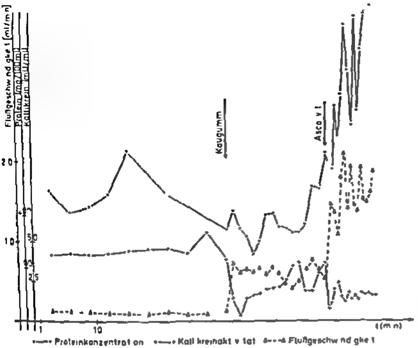

Abb. 7. Proteinkonzentration und Kallikreinaktivität im Parotisspeichel in Abhängigkeit von Zeit und Stimulationszustand

kommenden Kininogen pharmakologisch aktive Kinine ab. Dabei unterscheiden sich Serum- und Speicheldrüsen-Kallikrein. Speicheldrüsenkallikrein bildet Kallidin, Serumkallikrein Bradykinin. Die Kinine senken den Blutdruck, erhöhen die Kapillarpermeabilität und wirken schmerzerzeugend.

Die Untersuchung dieses Enzyms ist zur Erkennung von Entzündungszuständen von Interesse. Bei einem drüsenspezifischen Enzym sollten darüber hinaus bei chronischen Prozessen Aktivitätsveränderungen zu erwarten sein. Die Bestimmungsmethode der Kallikreinaktivität (Spaltung eines Argininesters) läßt bei Verwendung spezifischer Inhibitoren darüber hinaus Aussagen über das Auftreten weiterer Proteasen zu. Das Vorkommen normalerweise nicht vorhandener Proteasen oder die mangelnde Hemmung körpereigener Proteasen ist möglicherweise Indiz für prognostisch ungünstig verlaufende Entzündungen.

Die Abbildung 7 zeigt den Abfall der Kallikreinaktivität mit steigender Flußgeschwindigkeit bei einer Person. Die Kurve zeigt Ähnlichkeit mit der Änderung der Lysozymaktivität. Die Aktivitätsmessungen bei 100 Personen zeigen im Mittel einen Abfall nach Stimulation auf 10–20% der Ruhesekretion bei gleichzeitiger Erhöhung der Sekretionsleistung auf das 2–3 fache. Der Zusatz von Sojabohnen-Inhibitor, der alle körpereigenen Proteasen außer Kallikrein hemmt, verändert bei Normalpersonen die Aktivität nicht. Keine Aktivität ist meßbar, wenn nach Zugabe von „Kunitz-Inhibitor" aus Rinderlunge auch Kallikrein gehemmt wird. Die esterspaltende Aktivität des Speichels beruht bei gesunden Personen auf seinem Gehalt an Kallikrein. Diese Untersuchungen werden auf Patienten mit Erkrankungen der Parotisdrüse ausgedehnt.

RÉSUMÉ

L'activité des enzymes amylase, lysozyme, kallikreine qui sont spécifiques pour les glandes salivaires, est étudié touchant sa dépendance de la vitesse du flux. La détermination des variations normales concernant des états différentes de la sécrétion est favorable. Contrairement à l'activité de l'amylase, l'activité du lysozyme et du kallikreine ■ diminuit fortement quand la vitesse du flux s'augmente. L'activité secreté, par unité du temps, croît.

La comparaison de l'activité des enzymes de la salive

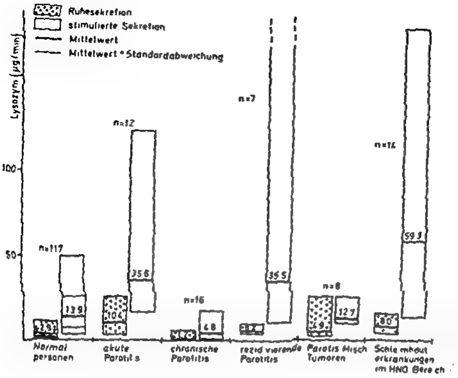

Abb. 5. Mittelwerte und beobachteter Bereich der Lysozymsekretion eines Normalkollektivs und bei Erkrankung der Parotis vor und nach Stimulation

Lysozymaktivität bei normaler Flußgeschwindigkeit und Proteinkonzentration deutet auf eine allgemeine Steigerung der Lysozymsekretion der Parotis bei entzündlichen Prozessen r Schleimhaut hin

Ungeklärt ist bisher, ob die Erhöhung der Lysozymaktivität bei bestimmten pathologischen Prozessen auf eine vermehrte Synthese in den Drüsenzellen oder auf steigendes Eindringen von Leukozyten in das Drüsengewebe zurückzuführen ist. Dabei steht der Nachweis über das Vorliegen von Isoenzymen und ihre Trennung noch aus

Abb. 6 zeigt die elektrophoretische Trennung im Bereich der basischen Speichelproteine und die Fraktionen mit Lysozymaktivität. Ob es sich bei den 3 Fraktionen um Isoenzyme handelt, kann noch nicht entschieden werden

KALLIKREIN

Kallikrein (EC 3.4.4.21) gehört zu den Peptidylpeptid-Hydrolasen, zeichnet sich aber durch eine hohe Substratspezifität aus. Es spaltet aus dem in der Globulinfraktion vor-

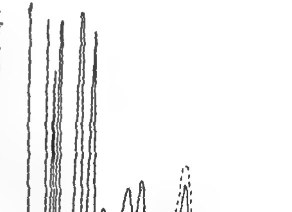

Abb. 6. Polyacrylamidgel Elektrophorese von Parotisprotein mit Lokalisation der Lysozymaktivität

Acta Otolaryngol 81 323–329, 1976

ACT OF SWALLOWING IN THE FIXED LARYNX

Z Krajina and S Večerina

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Abstract In a 16-year old female complete stenosis of the larynx and hypopharynx developed as a consequence of the ingestion of lye cristals Supraglottic laryngectomy plastic surgery of the hypopharynx and antethoracic oesophagus were performed and the upper aero-digestive tract reconstructed The dilated larynx remained fixed by the surrounding fibrous tissue but the sphincteric action of the mobile vocal cords was partly salvaged In that condition the act of swallowing could be reestablished The pathophysiology of this new mechanism of swallowing is discussed

The act of swallowing is a complicated neuromuscular mechanism New methods of examination, such as electromyography cinematoradiography and intraluminal manometry contribute daily towards a better knowledge of this mechanism Understanding of the act of swallowing may be complemented by a better knowledge of the compensatory mechanisms of swallowing which develop after mutilating surgical interventions or as a result of various functional disorders

Precisely for this reason we decided to analyse the act of swallowing in a female patient with severe functional and morphological changes in the aero digestive tract by making use of modern methods

NORMAL ACT OF SWALLOWING

On the basis of previous experience and the latest investigations the division of the swallowing act into the oral the pharyngeal and the oesophageal phase has today been generally accepted Only the first phase is voluntary i e controlled by the patient s will the second and third phases are reflex phases and begin at the moment when the bolus prepared by the masticatory musculature in the oral cavity produces excitation of the pharyngeal receptors and thus starts an entire train of reflex muscular contractions of the pharynx, larynx and oesophagus During the reflex phase several actions intended for the protection of the larynx take place At the moment when the bolus reaches the base of the tongue the larynx begins to rise Displacement of the base of the tongue dorsally moves the bolus to the pharyngeal constrictors which by their contraction tend to push it towards the oesophagus The forward and upward movement of the larynx is accompanied by a lowering of the epiglottis contraction of the vocal and the ventricular sphincter and the sphincter of the aryepiglottic folds After the laryngeal vestibulum has completely narrowed, the entire larynx tends to slant backward up to 90° Immediately afterwards, i e when the pharyngeal constrictors have reached their maximum contraction the crico pharyngeal sphincter suddenly relaxes and a propulsion of the bolus into the oesophagus ensues It is moreover considered that the forward movement of the larynx contributes towards the accessibility and the width of the crico pharyngeal sphincter at the time of relaxation

CASE REPORT

A girl aged 16 swallowed a considerable quantity of sodium hydroxide (caustic soda,

Fig 1 Laryngomicroscopic picture of reconstructed larynx showing the condition following supraglottic laryngectomy and removal of the fibrous membrane

lye cristals) with suicidal intentions Due to extensive corrosive changes which had affected the aero digestive tract tracheostomy nd gastrostomy were performed in another ical institution and shortly afterwards stic reconstruction of the larynx was at mpted by means of a free skin graft In spite of the application of a dilator the larynx repeatedly showed stenosis

On arrival in our clinic attempts were first made to identify the lumen of the oesophagus but neither oesophagoscopy nor radiology were successful apart from the fact that retrogradely via the gastrostoma it was established that oesophageal lumen was preserved only in the caudal third With the aim of restitution of the respiratory tract as the first operative intervention supraglottic laryngectomy was performed at our department The entire supraglottic part of the larynx was markedly stenosed and the scarred tissue had entirely obliterated the supraglottic anatomical structures The anatomical integrity of vocal cords and arytenoids was preserved The attempt to find the lumen of the oesophagus in the course of this operation was abortive too In view of the fact that all examinations showed complete obliteration of the greater part of the oesophageal lumen the indication was given for antethoracal plastic surgery ad modum Judin A pharyngostoma was subsequently formed and plastic surgery of the cervical part of the oesophagus undertaken making use of the rotating flaps

However in spite of the previously supraglottic laryngectomy the lumen of the larynx closed again Laryngomicroscopy showed the presence of a fibrous membrane which completely closed the laryngeal orifice After excision of this fibrous membrane and application of an acrylate dilator over a period of 10 weeks we succeeded in completely restoring the respiratory tract and decannulation was undertaken Repeated plastic surgery aiming at reconstruction of the cervical part of the oesophagus resulted in the forming of a continuous digestive tract starting with the oral cavity

Fig 2 Laryngomicroscopic appearance of the reconstructed larynx at the time of phon n Mobile arytenoids lead to partial closing of the rima g d

Fig 3 Microscopical picture of the orifice of the newly formed oesophagus The orifice of the oesophagus is shown *(left foreground)* The orifice of the larynx is distinguishable *(right background)* These two lumina are separated by a high mucosal fold

Laryngomicroscopy and hypopharyngoscopy undertaken after all these surgical procedures showed a broad laryngeal lumen (Fig 1) entirely fixed to the posterior pharyngeal wall and to the adjacent structures A certain mobility of the arytenoids was preserved however, so that on attempting phonation and during the act of swallowing the arytenoids moved and incompletely closed the rima glottidis (Fig 2) The orifice of the newly formed oesophagus was placed somewhat in front and to the left of the reconstructed oesophagus It had a diameter of approximately 1 cm and the sharp border between these two lumina was a high mucosal fold (Fig 3)

Although after restoration of the digestive and respiratory tract the patient experienced certain difficulties on swallowing because aspiration of food nevertheless occurred with the passing of time compensatory mechanisms developed and made possible a smooth act of swallowing In order to gain a better understanding of the act of swallowing in such extraordinary anatomical conditions we undertook photofluorography (4 pictures per second) of the act of swallowing and electromyography of the oral and the pharyngeal phase of swallowing

Photofluorography of the act of swallowing showed that neither in the erect nor in the Trendelenburg position was there any aspiration of the contrast meal and that it passed directly from the oral cavity to the left side of the hypopharynx that it was propelled into the newly formed oesophagus and that the contrast passed without hindrance though con

Fig 4 Profile photograph of the act of swallowing along with a presentation of the nasal cavity oropharyngeal structures and attachment of the jejunal fold The photograph shows a markedly high position of the tongue which is adhering to the hard palate The contrast meal normally passed the oropharyngeal structures with tortuous overflowing of contrast in the region of the pharyngo-jejunal junction The mucosal fold is visible at the level of this junction guiding the contrast meal into the attached jejunal fold

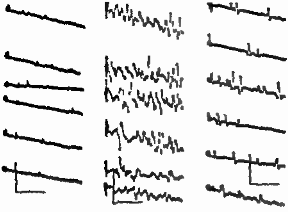

Fig. 5 Electromyographic registration of the action potentials obtained in the phase of swallowing (*a*) sparse action potential in the pharyngeal musculature (*b*) interfering innervation sample in the soft palate (*c*) action potential of the interarytenoid muscle

siderably retarded in relation to the physiological contrast emptying time An X ray study of the act of swallowing also confirmed a normal, synchronous and successive passage of the contrast meal along with normal passage over the mucosal fold between the larynx and the orifice into the newly formed oesophagus, without X-ray signs of regurgitation and with an oblique and dorsal position of the trachea ig 4)

hotofluorography and direct inspection of movements of the soft palate and the aryngeal walls during swallowing indicated anteposition and dropping of the soft palate contraction of the posterior pharyngeal wall lowering of the palatal arches altered by scarring of the tissue and marked raising of the base of the tongue

In this way the contrast meal was even in the oral phase directed ventrally towards the anteriorly placed orifice in the newly formed oesophagus which was separated from the larynx by an anatomical structure in the form of a staircase visible on all profile photofluorographic pictures

By testing the sensibility of the pharyngeal mucosa, hypo-esthesia was demonstrated i e practical anaesthesia of the pharyngeal wall disappearance of the pharyngeal and the palatal reflex and failure to distinguish sensory qualities in the posterior third of the tongue

Electromyography of the posterior pharyngeal wall helped us to obtain individual high frequency potentials while spontaneous activity was missing During phonation an intermedial sample appeared in the soft palate while at the moment of swallowing an interfering innervation sample with action potentials mostly of n rmal parameters was achieved Contraction of the posterior pharyngeal wall and the soft palate was simultaneous and in the second, so called pharyngeal, phase of swallowing we registered moreover action potentials of mostly normal parameters from the interarytenoid muscle Electromyography of the oropharyngeal swallowing phase registered a relatively small number of motor units in the pharyngeal wall while the electromyographic finding of the soft palate indicated maximal activation of the muscle elements in the act of swallowing (Fig 5)

Simultaneously carried out photofluorography of the act of swallowing and laryngography by means of a contrast spray indicated in this case a mutual relationship of the respiratory and the digestive tract In its upper pharyngeal part the digestive tract was placed laterally in front of the larynx which at the moment of swallowing did not change its position but remained dorsal in relation to the reconstructed digestive tract A divergence of the digestive and the respiratory tract occurred in this way (Fig 6)

DISCUSSION

For a normal act of swallowing full functional and anatomical integrity of all structures connected with the act is necessary However on account of the existence of a number of compensatory mechanisms the act of swallowing may nevertheless be restored in a certain number of patients with mutilated anatomical structures of the digestive tract Various reports have been published in the literature about the extent to which it is possible to

Fig. 6. A study of the act of swallowing along with presentation of parts of the laryngeal structure and the cranial part of the trachea by means of a contrast spray. This study shows a normal synchronous and successive passage of the contrast meal through the mucosal fold lying between the larynx and the orifice of the newly formed oesophagus without X-ray signs of regurgitation. The oblique and dorsally lying trachea is visible and the divergence of the trachea and the pharyngo-jejunal junction too.

restore the act of swallowing and how to set about the restoration in such pathological conditions. Simultaneous with the development of radiographic and electromyographic methods for the study of the act of swallowing some new observations have been made about these compensatory mechanisms. Staple & Ogura (1966) examined 36 of their patients by X-ray and cinematography following supraglottic laryngectomy and found that aspiration was present in 50% of all cases. According to these authors the following conditions must be fulfilled for the prevention of aspiration: complete occlusion of the vocal folds, mobility of the larynx and good accessibility to the oesophagus. Litton & Lenard (1969) found food aspiration in 16 of their 24 cases of subtotal laryngectomy. They too emphasize the importance of laryngeal mobility for the prevention of aspiration and the need for total laryngeal occlusion and mobility of the tongue. Calcaterra (1971) mentions that aspiration occurs after supraglottic laryngectomy because of loss of sphincter action of the larynx, limited laryngeal excursions and damage to the laryngeal sensibility due to injury of one or both laryngeal nerves.

In the case of our patient this particularly important protective role of the larynx in the normal act of swallowing was considerably damaged. The epiglottis and the vestibular sphincter were removed by supraglottic laryngectomy, the reduced mobility of the arytenoids could not safeguard a total closing of the rima glottidis and the reduced sensibility of the pharyngolaryngeal mucosa could also have contributed to the possibility of aspiration. Besides such a totally unprotected larynx was fixed to the adjacent tissue and the posterior pharyngeal wall so that the mobility of the larynx as one of the most important factors in its protection was not of much use.

It appeared that the central problem in the

interpretation of the mechanisms which developed in these extraordinary anatomical and functional conditions was to discover a new protective laryngeal *mechanism*

Photofluorography and electromyography of the act of swallowing and inspection of the movement of the oropharyngeal structures during the act of swallowing helped us to establish the presence of sphincteric formations in the region of the isthmus faucium The most important role in the forming of this sphincter is played by anteposition and dropping of the soft palate, considerable anteposition of the pharyngeal wall and raising of the base of the tongue In this manner the bolus becomes ventrally directed in relation to the dorsally lying respiratory tract No doubt in the smooth act of swallowing in our patient an important role was played by the connective mucosal fold formed on the border of the larynx and the orifice of the newly formed oesophagus as well as the partly preserved sphincter action of the vocal folds

Bosma (1967) described the so called ventral path of the bolus in the oropharyngeal phase of swallowing and considers that this is an entirely normal physiologic mechanism in newborn babies

Hypo-esthesia of the mucosa and the reduced number of motor neurons in the pharyngeal wall and despite this strong contraction of the palatopharyngeal structures in the phase of swallowing indicate that regardless of corrosion and numerous operations the important neuromuscular reflector pathways nevertheless remained undamaged

CONCLUSION

After complicated plastic surgery interventions we succeeded in completely reconstructing the aerodigestive tract following considerable corrosive injury to the larynx hypopharynx and the oesophagus Functional restitution of most of the damaged anatomical structures followed anatomical reconstruction Although the protective role of the larynx in the classical sense was missing altogether in this case the act of swallowing was functioning smoothly without aspiration We consider that anteposition of the soft palate and the so called ventral path of the bolus are contributory factors, as also are the partly preserved activity of the vocal fold and the arytenoids the existence of connective mucosal folds at the edge of the larynx and the mouth of the oesophagus and the dorsal location of the upper respiratory tract in relation to the forward displaced *hypopharynx and* oesophagus It seems therefore that the dorsal location of the larynx and its complete fixation to the posterior pharyngeal wall completely preventing its forward movement were favourable in this case too This example also indicates the numerous possibilities of the compensatory mechanisms in man even following serious anatomical and functional injuries Although all classic compensatory mechanisms were entirely missing in this case a new mechanism developed Accordingly the statement that swallowing is impossible if the larynx is fixed cannot be considered to be the rule without exception

RÉSUMÉ

Après avoir pris de cristaux de la soude caustique une stenose complète du larynx et de l'hypopharynx c'est développée chèz une fille de 16 ans Une laryngectomie supraglottique des plastiques de l'hypopharynx et l'oesophage antethoracal ont étaient faites et le tract aéro-digestif était reconstruit Le larynx dilatée restait fixée par tissue environnant mais tout de même l'action sphincterique des cordes vocales restait protégés De cette manière l'acte de la deglutition était rectablit La pathophysiologie de ce nouveaux mécanisme de déglutition est discutée

ZUSAMMENFASSUNG

Bei einem 16jährigen Mädchen kam es zur kompletten Stenose des Larynx und Hypopharynx die sich als Folge der Einnahme der Laugenkristalle entwickelte Eine supraglottische Laryngektomie Plastizierung des Hypopharynx und Formation des antethorakalen Oesophagus waren durchgeführt und auf diese Weise wurde der obere aero-digestive Weg rekonstruiert Der dilatierte Larynx blieb durch umkreisende fibröse Gewebe fixiert doch die sphinkterische Funktion der mobilen Stimmbänder wurde erhalten Auf diese Weise konnte der Schluckakt wieder

hergestellt werden. Die Pathophysiologie dieses neuen Mechanismus des Schluckaktes wurde diskutiert.

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DISCUSSION

D. F. N. Harrison: a) After all the considerable amount of reconstructive surgery is the residual larynx still functioning for phonation? b) Was it essential to carry out a supraglottic laryngectomy for long term investigation? c) Subcutaneous colon is less satisfactory than retrosternal since the latter is the shortest rante. The cosmetic effect of the subcutaneous colon may lead to further suicide attempts later in life. d) Finally, and relating to all the previous questions—how long ago was the last operation?

M. S. Karatay: As we know well a case loosing his epiglottis in time observed this type of cases in sy without any trouble in swallowing. But when we do operate for horizontal pentral operation for cancer we always observe some difficulty in swallowing which may last for a long time. We believe that this has to do with superior laryngeal nerve if preserved or the patient's age.

I want to ask from this point of view did you save the superior laryngeal nerve or did you cut it during the operation?

Z. Krajina (Reply) to Mr *Harrison:* We had to perform supraglottic laryngectomy because it was the only way to reconstruct the laryngeal lumen. All the supraglottic structures were highly deformed by the adjacent scar tissue.

Investigating the phonatory phenomenon in this patient we made a spectral analysis of the voice. The noise component predominated in sonagram, but a certain intensity of the voice was achieved because of the partially preserved function of the vocal cords.

We worked in collaboration with surgeons and they suggested antethoracal surgery after Judin. However, we had some difficulties in reconstructive plastic surgery of the cervical part of the oesophagus.

To Mr *Karatay:* During supraglottic laryngectomy we could not identify superior laryngeal nerve because of the abundance of scar tissue. We agree that it is very unusual that no aspiration occurred in such an unprotected larynx and that is why we tried to find out new pathophysiological mechanism of the swallowing in this case.

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MIDDLE EAR TRANSMISSION LOSSES CAUSED BY TYMPANIC MEMBRANE PERFORATIONS IN CATS

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Abstract Losses (determined by measuring microphonic responses) were essentially identical regardless of whether produced by admittance of sound into the middle ear via perforations in the tympanic membrane (TM) or in the bulla With SPLs monitored directly at the TM losses increased at 12 dB/oct with inverse frequency for $f<1$ kHz a pattern determined by the changes in the (calculated) net sound pressure acting from front and back on the TM Clinically hearing losses due to TM perforations are frequency-independent The difference between the two situations has mainly to do with the use of precalibrated SPLs in Clinical Audiometry

An earlier experimental study in cats (Payne & Githler, 1951) had suggested that perforations the tympanic membrane (TM) produce *equency independent* response losses, increasing in magnitude in some proportion to *the size of the perforation* (Fig 1) This result *is contrary to what one should have expected*

A perforation must leak some sound energy through to the backside of the TM to partially cancel the effect of the energy acting on its front, a notion expressed for example by Wever & Lawrence (1954) increased back pressure" Kobrack (1959) had likened the situation to that of a loudspeaker without a baffle where there is cancellation around the rim between the sounds radiated from front and back 'lost baffle effect' Such cancellations *depend on wavelength* In particular, below a speaker s resonance point the total radiated sound pressure falls off with 12 dB/oct toward lower frequencies With a perforated TM, there should be a similar frequency dependence of sound energy received by the ear In fact, Bekesy (1936) measured the *amplitude of mallar displacements in a human* cadaver ear and, after TM perforation, found it to decrease with inverse frequency at a rate of approximately 9 dB/oct The cut off frequency happened to be 400 Hz This latter *frequency should vary with the size of the* perforation, since the resistance of an opening increases with the flow velocity through it i e , with frequency in the case of in ac event

One potential flaw in the Payne & Githler study was seen in their *experimental method* They employed the changes in cochlear microphonic (CM) responses induced by the *perforation as their measurement criterion* In order to put the active electrode on the round

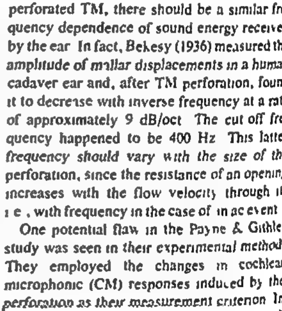


Fig 1 Attenuation of cochlear microph responses caused by tympanic membrane perfora ns in cats Parameter percentage of tympanic memb ane removed (*Figure from Payne & Githler 1951 Arch Laryng* 54 666)

This research was supported by several NIH grants

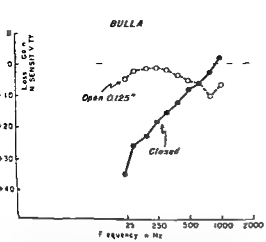


Fig 2 Cochlear microphonic response losses caused by a small tympanic membrane perforation with the bulla open (O) and closed (●) open system averaged results from three cats (Figure from McArdle & Tonndorf 1968 *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 192* 145)

window, they opened the cat's auditory bulla and then *kept it open throughout the experiment* With an open sound system (loud speaker) some sound must have already reached the backside of the TM before it was even perforated, providing a wrong baseline for the experiment

The first of our two studies (McArdle & Tonndorf, 1968) was undertaken in an effort to look at these objections Cats were again used Sound pressures necessary to produce a criterion CM response of 10 μv (round window electrode) were measured directly in front of the TM with the aid of a calibrated probe microphone (B & K, type 4134, 1 mm probe), before and after perforation A second probe microphone was inserted into the middle ear, directly behind the TM Perforations of uniform size (2 0 mm² in area) were set, usually in the posterior-superior quadrant, with the aid of a micro plug cutter, fashioned from a 16 gauge hypodermic needle

Fig 2 shows the response loss in terms of changes in sound pressure levels (SPL) in front of the TM, caused by a TM perforation (*a*) in the presence of a bullar opening, 3 ▮ mm (0 125") in diameter, and (*b*) with the bulla sealed off by a cotton wax plug An open system (loudspeaker) was used The difference between the two curves is obvious With the bulla open, the loss was nearly independent of frequency, as Payne & Githler had observed, while with the bulla closed the loss increased toward lower frequencies with 10 2 dB/oct, below a cut-off point of 1 kHz

Meanwhile, other experiments had shown that the cotton wax plug interferes with the dc pressure equilibration in the middle ear and does not produce a reliable seal of the bullar wall either We therefore changed our technique and began resealing bullar openings with dental cement, save for a short, sturdy walled, capillary tube that permitted dc pressure

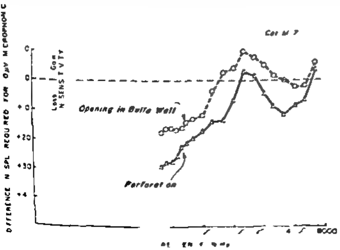


Fig 3 Cochlear microphonic response losses due to openings in the bullar wall (O) or in the tympanic membrane (●) open system results from one animal (Figure from McArdle & Tonndorf 1968 *Arch Klin Exp Ohren Nasen Kehlkopfheilkd 192* 145)

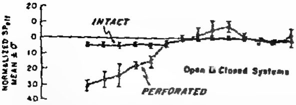


Fig 4 Effective sound pressure (SP_{eff}) across the intact and perforated tympanic membrane for a constant input combined results for open and closed systems averages of six cats 1 S D as indicated (Figure from Kruger & Tonndorf 1975 *J Acoust Soc Am* In press)

equilibration, but did not measurably transmit audiofrequencies (Tonndorf & Khanna, 1968)

Our second study on TM perforations (Kruger & Tonndorf, 1975) employed this latter technique, and the slope of the loss curve in the low frequencies became now exactly 12 dB/oct (cf Fig 7 below) The earlier value of 10 2 dB/oct (Fig 2) had apparently been due to some leakage via the cotton wax plug

That the frequency-dependent loss observed when the bulla was closed was indeed due to the sound energy reaching the middle ear directly no matter by which path way, was demonstrated in a second experiment of the first study (McArdle & Tonndorf, 1968) Comparable small openings were made vely either into the bullar wall or into TM Fig 3 gives the difference curves, more relative to the situation bulla osed and TM intact (open system) There is good qualitative agreement between the two curves The separation between them became smaller as the length of the canal perforating the bullar wall was made shorter Resistance as may be recalled varies with the length of a given channel and a TM perforation represents an extremely short channel Part of the difference between the two curves might also have been attributable to the loss of receiving area of the TM after its perforation a point we will return to presently

It is clear from Figs 2 and 3 that when the *open* bulla situation is taken as the baseline a TM perforation merely increases the already existing leakage to the back of the TM and produces an additional now frequency independent, response shift However, when the bulla is kept closed there is a loss sloping down toward lower frequencies at a rate of 12 dB/oct without an apparent limit

As already mentioned, a second probe microphone had been inserted into the middle ear We first ascertained the fact that this microphone, located in one fixed position gave a representative sample of the SPL behind the entire TM, within the frequency range of interest both before and after perforation (Kruger & Tonndorf, 1975) We then calculated the *effective* sound pressure (SP_{eff}) acting upon the TM by forming the vectorial difference between the readings of the two microphones on either side

Fig 4 gives SP_{eff} for a *constant input at the TM* both before and after perforation With the TM intact, the curve was essentially frequency independent Its extrapolation indicates that even at zero Hz there would still be a differential pressure across the TM capable of displacing it After perforation the curve sloped down toward lower frequencies below a cut off point of 800 Hz at 12 dB/oct without an apparent limit Its extrapolation indicates that, at zero Hz, there could no longer be a differential pressure across the TM Both of these extrapolations are supported by fact The findings of Fig 4 suggest that the configuration of the response loss of Fig 2 (or of Fig 7) is due to the changes in SP_{eff}

Fig 5 shows the SP_{eff} ratio before and after

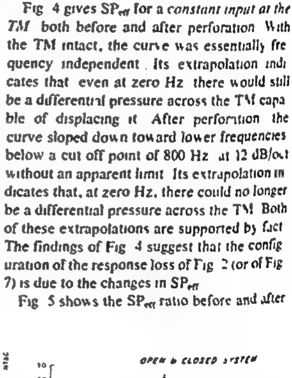


Fig 5 Ratios of effective sound pressure before after perforation) in reference to a constant cochlear-microphonic output combined results for open and closed systems average of six cats 1 S D as indicated (Figure from Kruger & Tonndorf 1975 *J Acoust Soc Am* In press)

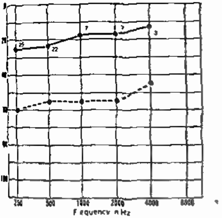


Fig 6 Conductive hearing loss caused by tympanic membrane perforations —— averaged data from 103 patients --- maximal individual loss (Figure from Anthony & Harrison 1972 *Arch Otolaryngol 95* 506)

TM perforation, but this time for a *constant CM response* This type of presentation, by definition, eliminates the 12/dB oct, low-frequency loss, but reveals a loss of approximately 10 dB that, in first approximation, is frequency-independent

The results of Figs 4 and 5 show (1) that the lost baffle concept is basically correct, and (2) that there is an insertion loss of approximately 10 dB, most likely caused by the loss in receiving area of the TM However, this latter loss is somewhat smaller than what should have been expected to result from a perforation of 2 0 mm² in area of a feline TM of 41 8 mm² (Wever & Lawrence, 1954) when the removal of the total TM produces a response loss of about 40 dB (Tonndorf & Khanna, 1968) The correct value should have been 13 6 dB

The problem was not yet solved for we had learned that audiometrically hearing losses caused by TM perforations tend to be *frequency independent* at least at f<kHz (Fig 6) Nevertheless, the apparent similarity with the findings of Payne & Githler had to be merely accidental, since in human patients there is no additional access route by which sound energy could directly reach the middle ear comparable to that through the bullar openings in the ears of experimental animals The situation was further complicated by the fact that in the tuning fork era TM perforations had generally been found to produce loss curves that sloped downwards towards lower frequencies (e g , Ostmann, 1909) Tuning fork tests were done in *open* systems, while electronic audiometers and their earphones employ *closed* systems

Realization of the discrepancy between our experimental animal data and those obtained audiometrically in human patients was what prompted us originally to set up the second study (Kruger & Tonndorf, 1975) The experimental technique of the new study was quite similar to that of the earlier one with a few exceptions

(1) The improvement of the bullar seal and its consequences have already been mentioned

(2) The sound was delivered either by a large overhead horn speaker (open system) or by a subminiature earphone (Knowles BP-1710 closed system) that allowed us to reduce the air space in front of the TM to approximately 0 7 cc We went deliberately to that extreme, since the earlier study had not shown any differences between open and closed systems, but the closed system then used had employed a relatively large air space of approximately 300 cc

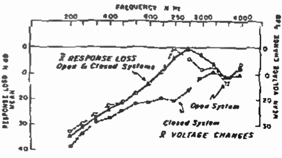
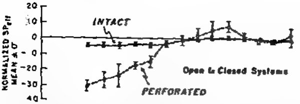


Fig 7 Cochlear microphonic response losses in terms of microphone readings in front of the tympanic membrane (mic II combined for open and closed systems (—) voltage changes across the transducer open system (O) and closed system (●) average of ten cats (Figure from Kruger & Tonndorf 1975 *J Acoust Soc Am* In press)



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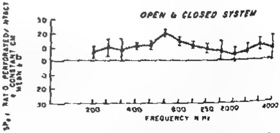

Fig 5 Ratios of effective sound pressure (before/after perforation) in reference to a constant cochlear microphonic output combined results for open and closed systems average of six cats 1 S D as indicated (Figure from Kruger & Tonndorf 1975 *J Acoust Soc Am* In press)

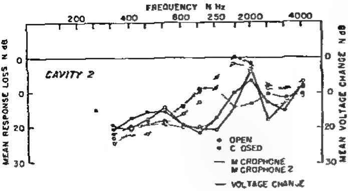

Fig 9 Cochlear microphonic response losses assessed at the entrance (mic 1) and at the bottom (mic 2) of an extended acoustically leaking ear canal (polyethelene tubing cm long) and associated voltage changes across the transducer see key at insert for the labeling of the various curves average of five cats (Figure from Kruger & Tonndorf 1975 *J Acoust Soc Am* In press)

Canal extensions of several lengths were fashioned from copper tubing (non leaking) and polyethelene tubing (leaking) The latter were included because ear canal and pinna show considerable leakage for audiofrequencies (Tonndorf et al 1966)

Consider first the results obtained with a non leaking canal extension (Fig 8) When assessed directly in front of the TM (mic 2) the response losses were virtually the same as those found with the shortened canal i e the curves had a slope of 12 dB/oct towards lower frequencies (cf Fig 7) When assessed at the canal entrance (mic 1) however the SPL losses became relatively larger in the midfrequency region giving the mic 1 loss curves a somewhat flatter shape At neither microphone position was there a significant difference between open and closed system data The *open* system voltage changes followed closely the SPL losses assessed at the canal entrance (mic 1) instead of those measured at the TM (mic 2) the situation found with the shortened ear canal (cf Fig 7) The *closed* system voltage changes deviated in the manner observed before (cf Fig 7)

With the leaking canal extension (Fig 9) the SPL losses measured at the canal entrance (mic 1) showed again relatively large shifts in the mid frequency region when compared to those assessed at the TM (mic 2) similar to what had been found with the non leaking canal (cf Fig 8) Once more there was no significant difference between open and closed system data for either microphone position However all loss curves now tended to flatten at their low frequency ends so that for the canal entrance position (mic 1) there was an essentially frequency independent region from 300 to 1250 Hz The SPL loss curves of Fig 9 are about as flat as the hearing loss curves of Fig 6 The voltage changes for both open and closed systems were similar to the corresponding data for the non leaking ear canal (cf Fig 8)

As was mentioned above tuning fork tests as a rule revealed hearing losses that increased in magnitude with inverse frequency Such tests employ an open system and give a measure of signal magnitude In terms of the present experiment changes in signal magnitude are represented by the open system voltage changes There is a suggestion in Fig 9 that the curve of the open system voltage changes did not become quite as flat at the low frequencies as some of the other curves (It must be added in this respect that the results of tuning fork tests do not lend themselves to a numerical assessment of the slopes of the hearing loss curves only the direction of such slopes positive or negative is evident)

In an effort to explain the results presented in Figs 8 and 9 we looked at the potential effects of canal resonances base line shifts etc but failed to come to a definite conclusion concerning their causes What is obviously needed is a rigorous network analysis

Although we are still lacking such an

analysis, the data of Figs 8 and 9 indicate that the frequency-independent low-frequency loss associated with TM perforations as observed in clinical patients has to do with (1) the presence of an ear canal that is leaky for audio frequencies, (2) the location at which the SPL measurements are taken, and (3) the use of precalibrated SPLs Essentially it is a matter of definition

RÉSUMÉ

Les pertes auditives (mesurees par les reponses microphoniques) étaient les mêmes quoi que soit la localisation de l entree du son dans l oreille moyenne (perforation tympanique ou ouverture de la bulla) Lorsque le niveau de la pression acoustique etait enregistre directement au niveau du tympan la perte de transmission était augmentee par 12 dB/oct on observait une frequence inverse par $f<1$ kHz la modification de la pression acoustique differentielle, agissant sur chaque coté du tympan montrait les memes traces En clinique les pertes auditives dues aux perforations du tympan sont indépendantes de la frequence du son La différence entre ces 2 situations est due essentiellement a l'emploi de niveaux de pression acoustique preetalonnés en Audiometrie clinique

ZUSAMMENFASSÛNG

Übertragungsverluste bestimmt durch Messung des Reizfolgestroms waren identisch gleichgultig ob sie durch Schalleintritt ins Mittelohr durch Perforationen im Trommelfell oder in der Bulla verursacht waren Wenn der Schalldruckpegel direkt am Trommelfell gemessen wurde n die Verluste mit 12 dB/oct und mit umgekehrter enz für $f<1$ kHz an Den gleichen Kurvenverlauf n die Anderung des (berechneten) Differentialschall ckes der von beiden Seiten auf das Trommelfell einwirkt Klinische Horverluste nach Trommelfell Perforationen sind frequenzunabhangig Der Unterschied zwischen den beiden Situationen ist hauptsachlich durch die Anwendung vorgeeichter Schalldruckpegel in der klinischen Audiometrie bedingt

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DISCUSSION

G Zechner 1) What influence has the diameter of the perforation? 2) Is there an influence by the location of the perforation? 3) Has an angled outer ear canal instead of a straight one any importance on the hearing level in your experiments?

R Hinchcliffe Have you looked into the possibility of other features that might account for the difference between the predicted and the actual form of the audiograms in man with eardrum perforation?

J Tonndorf (Reply) to Mr *Zechner* 1) We did not repeat the experiments of Payne and Githler with respect to the relative size of the perforation vs response loss We were satisfied with their results in that respect 2) There is a location effect the loss largest for a posterior perforation and this is in line with our concept of TM vibrations 3) Recall that we were mostly interested in frequencies <1 kHz and then the wavelength is so large that an angulation would have no effect

To Mr *Hinchcliffe* Sure traumatic perforations are more complicated than perforations carefully set in the laboratory Yet again frequencies <1 kHz such additional effects will be minimal and would not affect our results

Acta Otolaryngol 81 337–343, 1976

HEARING LOSS AND COCHLEAR PATHOLOGY IN MONKEYS AFTER NOISE EXPOSURE

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Abstract Old World monkeys were exposed to octave band noise from 0 5 to 8 kHz at 120 dB SPL 8 hours daily for 20 days Restricted permanent threshold shifts and localized loss of outer hair cells were produced which were reasonably well correlated with the exposure frequencies There was also a loss of both inner and outer hair cells at the extreme basal end of Corti s organ regardless of the exposure frequency Implications for human inner ear pathology are discussed

Personal experience and growing awareness of the long term ill effects of high level noise on human hearing have made psycho acoustic experimenters more cautious than they once were under the pressures of World War II (Davis et al , 1950) about exposing themselves or others to excessive levels of sound in the name of research In recent years cats (Miller et al 1963) and chinchillas (Carder & Miller 1972) have figured largely in such experiments where there was either risk or intention of producing permanent elevation of auditory thresholds (PTS) Both species can be trained for behavioral audiometry by avoidance conditioning and thus have the advantage over the notoriously refractory guinea pig The cat however has the disadvantage at least from the experimenter s point of view of being able to close his ear canals as a protection against unwelcome sounds (Hawkins et al 1943) The chinchilla on the other hand is extraordinarily susceptible to noise, and can be deafened by exposures having little or no permanent effect on the ears of other laboratory species (Drescher & Eldredge 1974) Extrapolation of data from the chinchilla to man requires a substantial and still undetermined correction factor lest damage risk criteria be set unrealistically low

Although squirrel monkeys have proved their value as subjects for investigating the temporary and permanent effects of noise on hearing and the histopathology of noise induced threshold shifts (Hunter Duvar & Elliott 1972) the Old World monkeys (Macaca and other genera) are more rugged and phylogenetically even closer to man When trained for behavioral audiometry by positive reinforcement (reward) conditioning (Stebbins et al 1973) they are willing to work for about an hour at a stretch producing reliable audiograms which differ from those of normal human subjects mainly in their slightly higher thresholds at 1 to 4 kHz and their extended upper limit of hearing (ca 45 kHz i e over an octave beyond the human range) Their organ of Corti shows a cytoarchitectural pattern somewhat less irregular but otherwise strikingly similar to that of the human ear (Johnsson & Hawkins 1967) We have found that they make highly satisfactory animal models for experiments on noise induced hearing loss S me correlations between noise exposure permanent threshold shifts and hair

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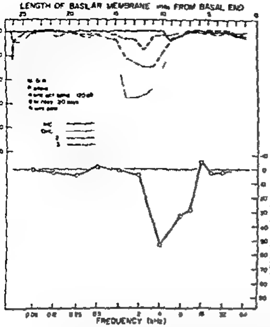

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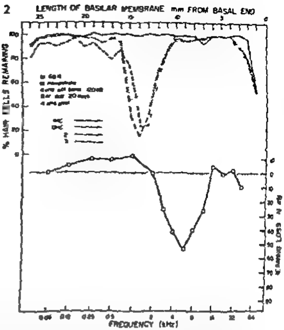

Figs 1–5 Below Final audiograms showing PTS in right ears of monkeys exposed to noise bands as indicated *Above* Hair cell losses from inner (IHC) and outer (OHC) hair cell rows for the same ears The placement of the logarithmic frequency scale with respect to the linear scale for the basilar membrane is arbitrary but corresponds reasonably well with frequency localizations determined in animals with abrupt high frequency losses produced by ototoxic aminoglycosides (Stebbins et al 1969 1973) Cf impulse-noise study by Pinheiro et al 1973

cell loss obtained in monkeys exposed to bands of noise at 117 to 120 dB SPL, 8 hours daily for 20 days, are to be reported here

METHODS

Subjects

Our subjects included members of the species *nemestrina mulatta* and *fascicularis* of the genus *Macaca* and the baboon *Papio papio* Their pre exposure audiograms were similar and no consistent differences in susceptibility to noise were found They varied in age from 2 to 9 years and in weight from 3 to 9 kg They were kept in individual cages and were given a restricted ration of food at the end of each day s session of audiometric measurements

Test equipment

Thresholds were determined after appropriate training (Stebbins et al 1973) with the animal seated in a primate restraining chair inside a double walled sound chamber (Industrial Acoustics Company) Calibrated ear phones (Permoflux PDR-600 with MX41AR cushions) were carefully fitted over the openings of the ear canals Each ear could thus be tested separately, and many of the problems of free field testing including the necessity of surgical de

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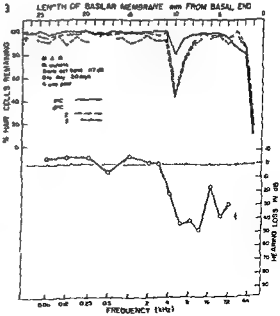

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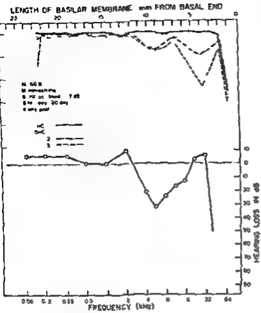

struction of one labyrinth (Miller et al , 1963) could be avoided The head was restrained, and positive reinforcers in the form of 190-mg banana-flavored pellets (Noyes) were delivered within easy reach of the tongue by a suitably placed chute

A hollow metal cylinder containing a red light was placed in front of the monkey at eye level Contact between his paw and the cylinder, which he was taught to maintain in the absence of sound stimulation, was detected by a circuit activated by a minute flow of current from the chair through the body and the paw to the cylinder Breaking this contact was the appropriate learned response whenever he heard a sound delivered to either ear

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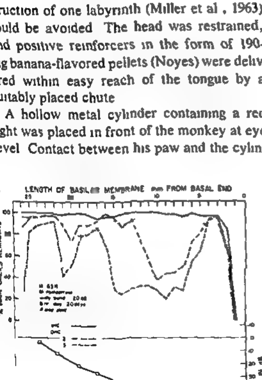

A small computer (PDP 8/L) was used to control the experimental contingencies and to record the data on punched paper tape for later off-line analysis Pure-tone stimuli were produced by a bank of nine oscillators (HP 204 C), attenuated by a programmable attenuator, and gated by a tone switch with a rise fall time of 50 msec

The ear phones were calibrated both by a 6-cc coupler and by a probe-tube microphone inserted through the cushion on the animal's ear so that the opening of the tube was directly in front of the ear canal

Exposure equipment

Noise exposures were made in an especially treated IAC room lined with 3 mm thick masonite and held in place by 25-mm² wooden struts placed randomly across the surface Noise was delivered by two Altec-Lansing 'Voice of the theater" speaker systems (511 B horn, 808 8A driver 416 8A low frequency speaker, N501-8A crossover), driven by a McIntosh MC 2105 power amplifier The noise was originated by a General Radio GR 1382 noise generator, and filtered by an Allison 2 BR variable band pass filter before amplification The octave bands produced were flat within ±5 dB, the wide band spectrum within ±10 dB from 100 Hz to 10 kHz

Training and threshold testing

Each animal was trained to touch the metal cylinder and maintain contact with it whenever the red light started flashing The light would then change from flashing to steady, and 1 to 9 seconds later a 2 5 sec trial was presented, which might be either a tone trial or a catch-trial One-third of the trials were of the latter kind, without a tone being

sounded If the monkey released his contact with the cylinder when the tone was presented, he received a food pellet If he responded to a catch trial, or when the tone was off, no reward was forthcoming, and the next trial was postponed for 5 sec to keep him honest

A release during tone presentation was counted as a correct detection, and caused the next tone to be attenuated by 10 dB Failure to release when the tone was on was counted as a miss, and caused the next tone to be increased by 10 dB This tracking procedure is a form of Bekesy audiometry It was continued until threshold had been crossed ("yes" to "no" and vice versa) ten times for each frequency A new frequency was then presented, and the procedure was repeated The SPL's of the last eight threshold crossings were averaged to determine the threshold Nine frequencies were tested daily for each ear

Noise exposure

Only after the thresholds had become stabilized to a criterion of less than 5 dB difference at any frequency for two of the last ree days was the noise exposure begun Two keys were exposed to each of four octave ds of noise centered at 0 5, 2, 4, and 8 kHz spectively, and two others to a wide band of noise (100 Hz to 10 kHz) Exposure levels were 120 dB SPL, except for the 8 kHz band, for which only 117 dB SPL was available The animals were exposed for 8 hours per day, 5 days per week, for 4 weeks i e for 160 hours in all, with rest periods of 16 hours per day and two days off on weekends They were tested after each daily exposure

Recovery

At the end of the four weeks of exposure, testing was continued for 30 days to allow whatever recovery might occur to take place and the permanent shift to become stabilized Daily audiometric testing was continued

Cochlear histological examination

When the final audiogram had been taken 30 days after the end of the exposure period the animals were anesthetized with pentobarbital sodium for sacrifice The membranous labyrinth was fixed and stained *in situ* by perilymphatic perfusion of 1 per cent OsO_4 solution (Zetterqvist) Cochlear tissues were prepared by microdissection (Hawkins & Johnsson, 1976*a*), and whole mounts (surface preparations) of the organ of Corti and basilar membrane were examined by phase contrast microscopy A complete count of the hair cells was made throughout the 25 to 27 mm length of the basilar membrane, and the percentage of hair cells remaining in each row per mm of length was plotted as a cytocochleogram In counting, the decision made was whether each cell was present or absent, *i e* replaced by a phalangeal scar No attempt was made to assess lesser degrees of change which might be thought to signify a decreased capacity to respond to sound stimuli

RESULTS

Threshold shifts

The threshold shifts measured after the first 8 hours of exposure reached maxima of 60 to 85 dB and were as great as or generally greater than any subsequent shifts recorded We may refer to these initial losses as compound threshold shifts (CTS) since they include both temporary (TTS) and permanent (PTS) changes in threshold The final PTS measurements, obtained 30 days after the last exposure were always less than the CTS but their maxima still averaged 40 to 55 dB depending upon the frequencies of the exposure band The 500 Hz band was least effective in producing threshold shifts as already indicated in data obtained from human subjects (Davis et al 1950) and from chinchillas (Carder & Miller 1971) Losses produced by the 500 Hz band and the wide band tended to be broader and less abrupt than those produced by the higher frequency bands All of the losses were essentially symmetrical for the two ears

Relations between CTS and PTS for the various exposures are described in greater detail elsewhere (Moody et al., 1975). Here we are presenting PTS measurements in one ear of each of four of the animals exposed to 4 kHz and 8-kHz octave bands, and in one exposed to the wide band of noise. Brains from all but one of these animals were sent to Dr. Jens Hall at the University of Oslo for study of possible neuronal degeneration in the cochlear nuclei, secondary to the hair cell losses in the cochlea. It was thought that they offered an opportunity to correlate circumscribed cochlear lesions representing well defined threshold shifts with presumably restricted areas of neuronal change in the central nuclei, and thus to obtain additional evidence for frequency localization at that level.

Correlation between PTS and hair cell loss

In M 51 and M 68, both exposed to the 4 kHz band, the PTS extends from 2 kHz to 16 kHz reaching a maximum at or just above 4 kHz, the center of the exposure band. The hair cell losses involve the outer hair cells mainly in the region from 9 or 10 to 16 mm from the basal end of the basilar membrane. In monkeys M-41 and M-66, which were exposed to the 8 kHz band, the hearing losses (PTS) are strikingly similar, involving at least the three octaves above 2 kHz. The maximum loss is at 4 kHz for M-66. In M-41R the loss is broader, with maxima at 4 kHz and 10 kHz, but the hair cell losses for the two are quite different. In M-66 the loss is greatest for the third row outer hair cells between 2 and 7 mm, whereas in M-41 the 7 to 11 mm area is chiefly affected.

In M-63, exposed to the wide band, there is a correspondingly wide range of frequencies affected, with a maximum loss around 8 kHz. Outer hair cells are absent chiefly from the third row and to a lesser extent from the other rows, from 4 to 17 mm, with a second area of loss centered at 20–21 mm.

A remarkable feature of all but one of these cochleas is the loss of hair cells at the extreme basal end of the organ of Corti, generally accompanied by a hearing loss for frequencies of 32 kHz or higher. The one exception is the baboon M 51, which exhibited another idiosyncracy, in that the loss of first row outer hair cells was greater than that for the second and third rows.

DISCUSSION

The correspondence between hearing loss and hair cell loss in these animals is reasonably good, although by no means so sharply defined as that in our animals with abrupt high-frequency losses from ototoxic aminoglycosides (Stebbins et al., 1969). It should be recalled that the positioning of the audiogram with respect to the cytocochleogram in the figures is arbitrary, since the audiogram is plotted on conventional four-cycle semi-logarithmic coordinates. The correspondences are at best approximate and no attempt has been made to indicate a more precise localization of frequencies along the basilar membrane in accordance with the results of our earlier study. If this were done the frequencies from 2 kHz to 45 kHz should be shown more widely spaced, occupying the lower 15 mm of the basilar membrane, with frequencies below 2 kHz crowded into the upper 10–12 mm of its length.

In M-41 and M-66, the animals exposed to the 8 kHz band, the hearing losses clearly involve more frequencies than can be accounted for by the relatively small areas of hair cell loss. It appears that hair cells which were recorded as present must have been affected by the noise—hardly a surprising conclusion, since there must presumably be gradations of injury to individual hair cells short of complete dissolution.

The permanent threshold shifts produced in these animals are not unlike the temporary shifts recorded in human subjects by Davis et al. (1950) after pure-tone exposures of shorter duration and equal or greater intensity. They do not, however, bear out the generally-accepted doctrine that the maximum loss is

necessarily one-half octave above the exposure frequency, nor was that always the case in the subjects of Davis et al Instead, our results suggest what might be termed a "central tendency", reminiscent of the familiar 4-kHz dip in the audiograms of patients with noise-induced hearing loss, regardless of the spectrum of the noise In other words, losses caused by exposure to lower and middle frequencies tend to be displaced upwards toward the region of maximal sensitivity, but losses for higher frequencies, such as 8 kHz, tend to be centered at the exposure frequency or to be displaced downwards

A new finding here is the loss of hair cells, inner as well as outer, at the basal end of the organ of Corti This loss would affect frequencies above the usual clinical audiometric range We have seen such a lesion in the inner ears of patients with noise induced hearing losses (Hawkins & Johnsson, 1976*b*) and have referred to it as the *juxtafenestral lesion* distinguishing it from the *tonotopical lesion* at 9–13 mm associated with the 4-kHz dip The juxtafenestral lesion tends to reaffirm the peculiar vulnerability of the sensory elements of the 'hook' region near the cecum vestibu re Exposure to everyday noise may help to ount for this type of lesion even in young rs (Johnsson & Hawkins 1972) with no ther evidence of cochlear pathology

RÉSUMÉ

Des singes du vieux monde ont ete exposes a des bandes de bruit blanc d octave entre 0 5 et 8 kHz a 120 dB SPL pendant 20 jours à raison de 8 heures par jour Ils ont manifeste des elevations limitees du seuil auditif et des pertes localisees des cellules ciliees externes correspondant assez bien avec les frequences d exposition Nous avons aussi observe une perte des cellules ciliees internes et externes au fond du premier tour quelles que soient les frequences d exposition Nous nous proposons d exposer les implications de ces observations pour la pathologie de l oreille interne chez l homme

ZUSAMMENFASSUNG

Affen der Alten Welt wurden 20 Tage lang 8 Stunden taglich mit Oktavbandrauschen von 0 5 bis 8 kHz bei 120 dB Schallpegel beschallt Der auftretende begrenzte

Man sah auch Verlust sowohl der inneren als auch der außeren Haarzellen am unteren Ende der Basalwindung unabhangig von der Beschallungsfrequenz. Die Folgerungen fur die menschliche Innenohrpathologie werden besprochen

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DISCUSSION

H Spoendlin The most striking finding in your experimental animals is the considerable hearing loss in spite of full presence of the inner hair cells You knew that there are several other reports where loss of outer hair cell alone did not affect the behavioral threshold The presence of inner hair cells does however not mean that they function normally and the point must be stressed that it is not sufficient to evaluate only the presence or absence of the cells without examination of their structural integrity After acoustic trauma with long term survival we found frequently the outer hair cells missing and the inner hair cells present however with considerable permanent ultrastructural changes of the receptor pole

In some of your animals there was no tonotopical correlation between hearing loss and hair cell loss How do you explain this?

In no se experiments with narrow band noise we found most damage in the middle frequency and more damage in the high frequency range was only found when sound intensity was given in dB/Hz How was it done in your experiment

R Thalmann In this context I would like to draw your attention to the recent findings of Dr Barbara Bohne which rather convincingly suggest that certain types of localized acoustic lesions of the organ of Corti may be due to the toxic action of endolymph In this case the acoustic trauma per se produces small holes in the reticular lamina while the major damage results from the endolymph leaking into the fluid spaces of the organ of Corti

D Hilding I would like to say a word on behalf of monkeys When planning research using primates I think we should ask ourselves certain questions 1) Is it essential in order to gain important information to use primates instead of other mammals? 2) Where will they come from? From colonies where they are bred for medical research—or are they kidnapped from the forests of South America or India 3) Will as much information as possible be obtained from each subject? For instance in this experiment Spoendlin s whole modern technique could probably have been used to leave open the possibility of electron microscopic examination of the organ of Corti

M Ross[1] (Reply) to Mr *Spoendlin* The outer hair cells were the ones chiefly missing in the present material We agree that subtle changes particularly in the inner hair cells could be important in contributing to the permanent threshold shifts noted According to the present method only missing hair cells were counted because the presence or absence of a hair cell is an objective observation

To Mr *Thalmann* No response to his interesting comments is required

To Mr *Hilding* (The paragraph relating to the reasons for the use of primates in this study was read from the manuscript) We agree with your general remarks concerning the need to conserve and protect primates and to make most extensive use possible of their tissues whenever primates are considered essential in an experiment

[1] In the authors absence the paper was read by Dr Muriel Ross of the Department of Anatomy University of Michigan

Acta Otolaryngol 81 344–352, 1976

THE COCHLEAR NUCLEI IN MONKEYS AFTER DIHYDRO STREPTOMYCIN OR NOISE EXPOSURE

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Abstract The cochlea and the cochlear nuclei in monkeys subjected to dihydrostreptomycin treatment or loud noise are investigated Their cell populations are compared with the findings in the normal monkey

In recent years the anatomy of the cochlear nuclei in different species has been the subject of increasing interest Their anatomical configuration and the number of their nerve cells are described in man, cat, whales, bats and seals (Hall, 1964, 1967, 1969, 1974) The development of the nuclei is described in mice (Mlonyeni, 1966), their cyto architecture nd their afferent and efferent connections are ribed in the cat and the porpoise (Osen & ısen 1965 Osen 1970) Thus our knowledge the normal anatomical appearance of these nuclei is increasing

At present research activity is also directed towards the influence of pathologic conditions on the cochlear nuclei As our knowledge of the influence of streptomycin dihydro streptomycin, asphyxia and noise on the hair cells of the cochlea increases it is only natural that investigations concerning pathologic conditions are extended to include the cochlear nuclei Windle (1961) Windle et al (1962) and Hall (1964) described the reaction of cochlear neurons to asphyxia Hansen (1973) showed that nerve cell changes occur in senility, and Mair (1973) showed that in hereditary deaf white cats the areas and volumes of the cochlear nuclei are no greater than in kittens The effects of noise on nerve cells are described in a paper by Liden et al (1973) and Hall (1974), in cats, and recently in the guinea pig by Theopold (1975)

Thus previous findings indicate that the various cells of the primary auditory nuclei have their own particular, specific way of reacting and then show the same reaction to various noxa such as injury, asphyxia, or antibiotics, and perhaps also to noise

The present investigation is an attempt to show whether noise which causes cellular degeneration in the cochlear nuclei of the cat but no loss of cells, will cause more obvious damage when inflicted upon another species the macacus monkey, which may be more sensitive In some monkeys the effect of dihydrostreptomycin upon the central auditory pathways was also investigated

MATERIAL AND METHODS

The monkeys were the same as those described by Hawkins (1968) Fourteen of his monkeys were included in this material Three *Erythrocebus patas* monkeys were given dihydrostreptomycin Five *Macaca nemestrina* 1 *Erythrocebus patas* and 1 *Macaca mulatta* were exposed to noise of various octave bands for periods varying from 2 to 160 hours and the remainder of the monkeys 3 *Macaca nemestrina* and 1 *Macaca mulatta* served as controls (Figs 1 2 3)

		NOISE EXPOSURE [illegible]			
No	SPECIES	EXPOSURE	HOURS	MONTHS POST EXP.	HEARING LOSS
M-46	E PATAS	2 kHz 110 dB	2	1 5	26 dB
N-6	M NEM	2 kHz 120 dB	40	2-3 HRS	83 [illegible]
M-6 [illegible]	M NEM	2 kHz 120 dB	10 MIN	2 HRS	NOT MEAS
M-68	M NEM	4 kHz 120 dB	160	1 3	50 dB
M-41	M MULATTA	8 kHz 117 dB	160	1	50 dB
M 66	M NEM	8 kHz 117 dB	160	1	40 dB
M-63	M NEM	N BE BAND 120 dB	160	1 1	60 dB

Fig 1 Experimental conditions and measured hearing in the noise-exposed animals

The hearing was tested in all monkeys before and after experimentation. The 3 *Erythrocebus patas* treated with dihydrostreptomycin were sacrificed 0.8, 4.5 and 3 months post exposure. Among the noise-exposed monkeys, one was exposed for only 10 minutes, and then sacrificed after 2 hours, another exposed for 40 hours and sacrificed after 2–3 hours, 2 others were sacrificed one month after exposure, and the rest 1.1, 1.3 and 1.5 months post exposure.

An appropriate dose of Nembutal was administered, the brain and the medulla removed and immediately placed in 10% Formalin.

As the cochlear nuclei are situated in the lateral corner of the IV ventricle on the surface of the medulla, the Formalin penetrates quickly and artifacts are not likely to occur. The blocks containing the cochlear nuclei were later immersed in 96% alcohol for 14 days. They were then dehydrated, embedded in paraffin and cut in transverse sections at 15 µm. Every 10th section was mounted and stained with thionine. Controls were treated in the same way as experimental animals. The method described by Hall (1964) was then applied for the study of the cells in the cochlear nuclei, and computation of the number of neurons.

		[illegible]			
No	SPECIES	[illegible]	[illegible]	SACRIFICED MONTH POST [illegible]	HEARING LOSS
[illegible]	E PATAS	[illegible] 100 mg/kg	[illegible]	8	[illegible]
[illegible]		100 mg/kg	9	4 5	[illegible]
[illegible]		100 mg/kg	75	3	[illegible]

Fig 2 Experimental conditions and measured hearing in the DHSM treated animals

		CONTROLS	
No	SPECIES	[illegible]	
M 36	M NEM	REAC ION TIME	----
M 01	M MULATTA	NORMAL	---
A 7	M NEM	IMPLANTED COCHLEAR ELECTRODE	----
A 8	" "	" " " [illegible] ATROPHY	10 dB

Fig 3 The controls

RESULTS

The nerve cells of the cochlear nuclei showed the same degeneration patterns as described in cats (Liden et al., 1973, Hall, 1974). Two types of degeneration, the pycnotic and the chromatolytic, occurred both in the noise exposed animals and in those treated with dihydrostreptomycin, but not in the controls (Figs 4, 5).

The degenerated cells were intermingled with more normal looking ones, and also with remnants of cell bodies vaguely seen, so-called "ghost cells" (Fig 6). An attempt was made to ascertain whether any of the different cell types described by Osen (1969) were especially susceptible. Generally speaking it was found that the pyramidal cells of the dorsal cochlear nucleus (Fig 7) were pycnotic, both in the noise-exposed and the DHSM treated animals. In the ventral nucleus the multipolar cells and the octopus cells in the ventral part of the ventral nucleus showed a vacuolysing cytoplasm with transitions to ghost cells, whereas the large and small spherical cells tended to be pycnotic. Thus, the general trend

Fig. 4. Ventral cochlear nucleus. (A) In a normal control. (B) DHSM-tested animal (M-44). Great loss of cells (×40).

was that the greater cells showed the chromatolytic type of degeneration, and the smaller ones tended to become pycnotic, but because of the multifarious picture, no definite statements can be made. Neither the vestibular nuclei nor the superior olivary nuclei (Fig. 8) showed degenerative signs.

The results of the cell counts are seen in Figs. 9 and 10. Fig. 9 shows the controls. On average the ventral nucleus contained 79 300 cells, and the dorsal nucleus 27 600 cells, in all, 107 400 cells. As the ventral cochlear nucleus is usually divided into an anteroventral and posteroventral part, this was also investigated in six nuclei from the controls.

These subdivisions were, however, not so

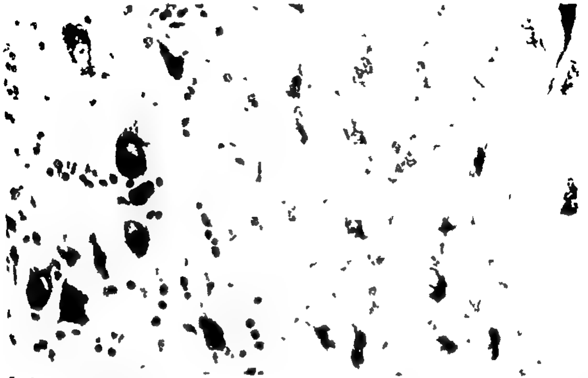

Fig. 5. Ventral c.n. Multipolar cells and octopus cells. Control: normal appearance (×500) and the same region from monkey M-67 showing vacuolyses, cytoplasm ghost cells and cell remnants (×500).

readily distinguishable in monkeys as they are in cat or man, as their borders and the different groups of cells were more intermingled. Therefore the division was not maintained in the experimental animals where degeneration of the cells further complicated the task. In the counted controls the anteroventral nucleus contained on average 47 400 cells and the posteroventral nucleus 32 500 cells.

The size of the nuclei was on average, 7 mm³. The results of the cell countings in the dihydrostreptomycin treated animals and the noise exposed ones are shown in Fig. 10. No average figures are given, as the experimental conditions differed both for each group and within the groups. However, it is seen that *neither* of the experimental animals reached *the* approximate average number of cells in the controls, and this holds true both concerning *the* ventral or dorsal nucleus seen individually and the total number of cells.

The sizes of the nuclei were also reduced. In some of the experimental animals the size reached the average size of the controls, 7 mm³, but the majority were smaller, the average being 5.5 mm³. This shrinking of the volume is proportional to the reduction in cell numbers as seen in Fig. 11. The straight line in this figure shows the average of the different values, computed according to the chi square formula.

DISCUSSION

(a) Dihydrostreptomycin

Dihydrostreptomycin was used in the treatment of tuberculosis, especially the tuberculous meningitis during the years 1947–57 when several reports concerning its toxic action on the hair cells of the cochlea practically proscribed its use (Hall, 1956). The dosage used was about 40 mg/kg. Thus the present monkeys were tested with very high dosages 100 mg/kg, corresponding to 5 g/day, if com

Fig 6 Ventral c n Normal and pycnotic large and small spherical cells intermingled with chromatolytic ones (M 62) (×200)

pared with a human However, the damage it inflicts is more related to the total dose than the single daily dose Even in that respect our monkeys received high doses, confirmed by the fact that two were deaf and one had a hearing loss of 70 dB The effect of dihydro streptomycin on the nerve cells of the cochlear nuclei is never described, but it seems reason able to believe that it may cause nerve cell destruction as well as hair cell destruction This assumption is supported by Hawkins (1968) who stated that the lesions caused by a related drug, kanamycin, were not confined to the organ of Corti, but might extend to the auditory nerve and perhaps beyond

This statement is substantiated in the present report, as the most severely affected DHSM monkey, M-61, had lost 51 3% of its cells on the right side and 47% on the left side The least affected one, M-62, had lost 29 and 29 5% of its cells Allowing for every possible error of the method to occur, this amounts to 10%

If 20 to 40% more of the nerve cells succumb, this proves that DHSM in large doses is fatal, not only to the hair cells of the cochlea,

Fig 7 Dorsal c n Pycnotic and a few chromatolytic pyramidal cells (M-68) (×400)

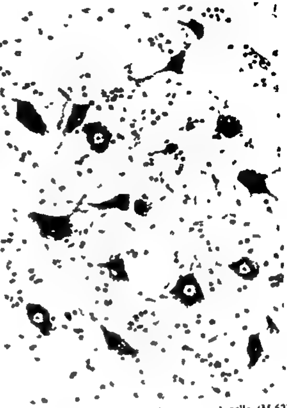

Fig 8 Superior olivary nucleus normal cells (M-62) (×300)

but also to the nerve cells of the primary auditory nuclei However, it remains to be seen whether smaller doses, more closely corresponding to clinical treatment will give the same results

(b) Noise

It is evident, from Figs 2 and 3 that in none of the noise-exposed animals was the total number of nerve cells preserved Those suffering from the greatest hearing loss (M 67 M-63 M-41) also had a substantial loss of nerve cells On the other hand those which had a milder loss of hearing (M-46, M-66 M-6886) also retained a larger number of nerve cells

The average was 83 400 or 22 4% varying from 34% (M-41) to 13% (M-46) Both figures are above the 10% allowed for the maximal amount of errors included in this method

The dorsal cochlear nucleus had suffered most On average 28% of the cells were lost

CONTROLS

MONKEY	SIDE	VENTRAL NUCLEUS	DORSAL NUCLEUS	TOTAL	SIZE (mm³)
A 8	R	87 200	23 600	115 800	6 4
	L	85 100	27 000	112 000	6 3
M-01	R	74 100	28 400	102 600	7 1
	L	77 300	28 800	106 100	7 3
A 7	R	TORN	24 600		
	L	79 600	[illegible] 500	111 100	6 6
M-36	R	71 5[illegible]	29 000	100 500	7 5
		[illegible] 600	22 800	103 400	8 1
AVERAGE		[illegible]9 300	27 600	107 400	7 0

Fig 9 The control animals the number of neurons and the size of the cochlear nuclei

	MONKEY	SIDE	VENTRAL NUCLEUS	DORSAL NUCLEUS	TOTAL	SIZE (MM³)	HEARING LOSS
DHSM	M 61	R	38 200	14 100	52 300	3.89	> 70 dB
		L	45 400	11 500	56 900	4 14	
"	M-44	R	43 800	27 900	71 700	3 71	DEAF
		L	49 100	27 700	76 800	4 3	
"	M-62	R	61 100	14 800	[illegible] 400	4 47	DEAF
		L	60 600	15 200	75 800	4 15	
NOISE	M-41	R	48 300	23 100	71 400	6 34	50 dB
		L	49 800	28 900	78 700	7 6	
"	M-63	R	53 200	22 500	75 700	6 6	60 dB
		L	64 500	16 300	80 800	7 2	
"	M-46	R	80 400	11 700	92 100	5 73	26 dB
		L	82 700	11 100	93 800	5 63	
"	M 66	R	62 100	26 200	88 300	6 02	40 dB
		L	70 800	21 800	92 600	5 99	
"	M-67	R	51 700	21 500	73 200	5 66	80 dB
		L	53 000	20 000	73 000	5 54	
"	M 68	R		CUT DURING PREPARATION			
		L	65 800	24 000	89 800	6 76	50 dB
"	M-6886	R	65 100	21 700	86 800	5 0	NOT MEAS
		L	69 100	20 300	89 400	5 0	

Fig 10 The experimental animals the number of neurons and the size of the cochlear nuclei compared with their loss of hearing

the ventral nucleus average being 21 % In one of the animals (M-46) the dorsal nucleus had lost 60 % of its cells This was an *Erythrocebus patas*, the so called military monkey from the Sudan, the same species as the DHSM treated ones The differing ability of the nerve cells to survive must be related to variations in the blood supply to the two nuclei It would also be interesting to see whether the loss of cells was related to the time elapsed after noise exposure In 5 of the monkeys this time was 1–1 5 months, whereas 2 of the monkeys were killed the same day In one of these (M-6886) exposed for 8 hours, only 16 % of the cells

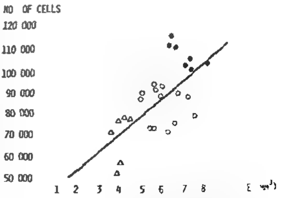

Fig 11 Volume of the cochlear nuclei compared to the number of neurons ▲ DHSM tested animals noise exposed animals ● controls

were lost which is not much above the variations which are implicit in the method In the other (M 67), exposed for 40 hours 32% This monkey had a hearing loss of 80 dB the highest measured The degenerations in this case *developed within 42 hours* This is a debatable point as no one knows how soon secondary nerve cell degeneration may develop To try to shed some light upon this question an electron microscopical investigation is also planned to see how soon after the influence of loud noise degeneration occurs in the cochlear nuclei

Of course individual variations in resistance blood flow and susceptibility of the nerve cells occur and that is why there are variations in the cell picture both between the various monkeys and between the dorsal and ventral nucleus in the same individual However as all the noise exposed monkeys showed a reduction in their cell numbers in the cochlear nuclei compared with the controls it may be stated that both dihydrostreptomycin and noise affects not only the cochlea but also the nerve cells of the primary auditory centres The present report does not claim to establish the reasons for the degeneration

CONCLUSIONS

Fourteen normal hearing Macacus monkeys were used for these experiments Four of them served as controls Three were given dihydrostreptomycin and 7 were exposed to noise of high intensity for a varying number of hours It is shown that both dihydrostreptomycin and noise affects the cochlear nuclei Two types of degenerations occurred either pycnotic or chromatolytic *in both of the experimental* series It is shown that the volume of the nuclei was reduced after experimentation and that a loss of nerve cells occurred more pronounced after dihydrostreptomycin than after noise The number of nerve cells was counted both in the controls and the experimental series and all of the latter showed a loss of nerve cells up to 50%

RESUME

Apres didydrostreptomycin ou de bruit fort une degeneration se trouve dans les cellules nerveuses du nucleon cochleaire chez les singes Macacus Ce complexe cochleaire s est retraite et le nombre de cellules nerveuses est fortement diminue

ZUSAMMENFASSUNG

Nach Dihydrostreptomycin Medikation oder schwerem Larm wurde Degeneration der Nervenzellen im Nucleus Cochlearis der Macacus Affen gefunden Der Cochlearis Komplex war geschrumpft und die Anzahl der Nervenzellen stark abgesetzt

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DISCUSSION

J Tonndorf My question is whether or not the poor correlation between the cochlear losses and the cochlear nucleus losses could be accounted for by the fact that the cochlea represents essentially a 2-dimensional structure while the nucleus is a much more complex 3 dimensional structure

H Spoendlin It would be essential to know whether ganglion cell degeneration in the cochlear nucleus are secondary to changes in the organ of Corti and retrograde degeneration of the primary neurons or if they occur independently Do you have more precise information of the correlation between the degree of ganglion cell loss in the cochlear nuclear and the alteration in the cochlea mainly loss of inner hair cells

R Hinchcliffe Very briefly was that curve on your last graph fitted in the method of chi squares?

J G Hall (Reply) to Mr *Tonndorf* You are quite right this is one of the difficulties As you will remember each fibre from the cochlea divides into branches to the anteroventral posteroventral and dorsal nuclei and make connections with hundreds of nerve cells in a three dimensional plane which give you a great problem when trying to decide any tonotopical organisation of the degenerated nerve cells

To Mr *Spoendlin* Yes there was a fairly good correlation between the number of nerve cells and the hearing deficiency Of course there are individual variations in the number of cells as well as deviations due to technical difficulties but largely seen it was like the asphyxiated children I described in 1964 The larger the asphyxia the more nerve cells lost In the present cases you find less nerve cells in those with longer noise exposition than in the shortly exposed ones

As to the problem of whether this is a secondary degeneration or something else I do not know I think there was a good comment in the latest number of Archives of Otolaryngology where Theopold one of Engstrom s pupils found degenerations in the cochlear nuclei of guinea pigs after heavy noise and stated that overstimulation causing a large energy consumption and a final breakdown of the cytoplasmatic metabolism might be responsible I do not know at present these are all theories

To Mr *Hinchcliffe* Yes the curve is computed according to the chi square formula

Acta Otolaryngol 81 353–363, 1976

MORPHOLOGICAL OBSERVATIONS OF IMMUNOBIOLOGY OF LARYNGEAL CANCER

Evaluation of the Defensive Activity of Immunocompetent Cells present in Tumour Stroma

O Sala and A Ferlito

From the Department of Otolaryngology University of Padua Padua Italy

Abstract A series of 104 patients with squamous cell carcinoma of the larynx was studied with regard to tumour–host interaction Prognostic evaluation was based upon histologic grading and morphological evidence of host immune response judged by the presence and degree of lymphocyte and plasma cell infiltration in tumour stroma Histologic grade and lymphoplasma cellular infiltration do correlate with the 5 year survival The immune response however seems to be a favourable prognostic sign only for well differentiated tumours in our series all poorly differentiated neoplasms showed minimal or no cellular response The survival rate increases with the increasing intensity of cellular response within each class of tumour cell differentiation Small lymphocytes are the basic elements of cell mediated immune response After tumour antigenic stimulation they change into immunoblasts which in turn would produce committed' lymphocytes which would recognize and destroy tumour cells

Criteria for the definition of the degree of malignancy and the course and the prognosis of a neoplasm have been either multiple or unique or more often associated Briefly they may be divided into three groups The first group of criteria pertains to the patient—age (it is a well known fact that a malignant tumour is more invasive and has a poorer prognosis in the young) the condition of the patient (a neoplasm is more aggressive and more rapidly fatal in patients in bad health) the coexistence of chronic infectious diseases (tuberculosis and syphilis seem to favour the development of a tumour) The second group of criteria pertains to the cancerous process proper its extension its microscopic aspect (exophytic, infiltrating), the anatomical site (it has been established that the prognosis is poorer when a neoplasm develops in certain sites, e g thyroid gland, hypopharynx, etc , even though an early diagnosis is made and the tumour is limited in size) and, finally, the presence of metastases to regional and/or distant lymph nodes

Actually the above criteria simply register established facts and do not aim at anticipating or preventing them This is why the attention of researchers and the hopes of clinicians have been drawn to a third criterion—the histological study of the tumour For this purpose the following characteristics should be mentioned the histological type of the tumour (Broders 1927), the histologic grading of malignancy (Jakobsson et al , 1973, Bloom, 1974) and the relationship between neoplastic tissue and the underlying stroma

Experience has shown that the clinical behaviour of cancer does not depend on the above criteria only, not even if considered in their broadest association In fact in human malignant diseases it may be noted that the same tumours arising in the same organs and having the same common factors (location, size histologic type etc) have a completely different biological and clinical behaviour, which at times may be also unpredictably favourable or unfavourable This means that any treatment even the most adequate may

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lead to different results This may well be due to the varying degree of the host s defen sive reaction against tumour The interaction between tumour and host—morphologically present with lymphoid inflammatory infiltra tion around the tumour—is extremely im portant for an understanding of the varying biological and clinical behaviour of the can cerous process

The malignant tumour is no longer to be considered as an invasive, diffusive auto nomous process, purposeless in its growth and independent from its host Burnet (1970) suggested that the development of a malignan cy may be related to a breakdown of the body's defensive mechanism to a defect in cellular immunity, to a failure in immunologic surveillance to a diminished unbalanced body s potential to control the neoplastic growth From this new approach it appears that when a cell becomes neoplastic, it ac quires new antigenic characteristics unknown to the host s immune system which must therefore learn to produce an adequate im mune response

A neoplasm develops when the body is unable to provoke this immune reaction to tumour The neoplastic cells escape the im ologic surveillance that is the body s ensive potential and the tumour can then row without being delimited and counter acted

The immune response against tumour is induced through the mechanisms of humoral and cellular immunity Humoral immunity is of lesser importance and is activated through the formation of tumour specific antibodies belonging to six classes of immuno globulins—IgG IgA IgM IgD IgE and IgND The latter class has been recently described by Johans son & Bennich (1967) These antibodies are effective by means of a toxic action on the tumour cell membrane

Cellular or histogenic immunity is by far the most important (Mackness & Blanden 1967, Burnet 1971, Starzl et al 1971 Laroye 1974, Marx 1974) and is mediated by lymphocytes, plasma cells and macrophages The latter are also actively cytotoxic to tumour cells

Recently, with regard to prognosis, most investigators have become increasingly con cerned in peritumoural stromal infiltrates composed of lymphocytes and plasma cells considered as an active, early cellular immune response

Recent investigations conducted on various organs, such as the breast (Hamlin 1968 Cutler et al , 1969), stomach (Inokuchi et al , 1967, Hawley et al , 1970), testis (Marshall & Dayan 1964) mediastinum (Marshall & Dayan, 1964), ovary (Marshall & Dayan 1964), kidney (Kiely et al , 1972), oesophagus (Takahashi, 1961), bladder (Sarma 1970 Tanaka et al , 1970) have demonstrated that the more marked is the lymphocyte and plasma cell infiltration in the tumoural and peritumoural stroma, the more favourable is the prognosis

This phenomenon must be regarded as a valuable cellular immune response as an ex pression of the body s reactive potential against tumour cells Many authors consider the stromal reaction as an antineoplastic phenomenon

Bennet et al (1971) carried out a study on 84 patients with squamous cell carcinoma of the larynx or hypopharynx and noted that the survival was longer for the well differentiated tumours and for those displaying dense lymphocyte and plasma cell infiltration and a marked germinal centre hyperplasia in regional lymph nodes In particular they found that of 81 patients with Grade I and II carcinoma the 5 year survival rate was 69% and 67% respectively while the survival of Grade III was 37% and Grade IV was 26% In their research the lesion had been classified int four grades of tumour cell differentiation—Grades I through IV

Considering the abundant tumour docu mentation at the Department of Otolaryngo logy of Padua University we thought it migh be helpful to conduct an investigation in orde

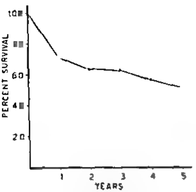

Fig 1 Observed survival of 104 patients with primary carcinoma of the larynx

to establish whether a causal relationship exists between lymphocyte and plasma cell infiltration around the tumour and a more favourable prognosis, taking into account the degree of tumour cell differentiation

MATERIAL AND METHODS

The present study was conducted by means of examination of 127 primary carcinomas of the larynx, but of 104 patients only we had sufficient clinical information, the remaining 23 having been lost to follow up 96 patients were males, 8 were females The age range of patients was between 36 and 83 years The study concerns only the patients hospitalized at our Department in 1968, so as to evaluate after a significant time lapse their survival

Those patients operated on or treated by X rays in 1968 who had previously undergone surgery for squamous cell carcinoma of the larynx were excluded from our series

Apart from 2 patients who refused treatment and 10 other cases treated by radiotherapy only the patients had undergone the following operations cordectomy, 18 epiglottidectomy 2 supraglottic laryngectomy 23 hemilaryngectomy 1 frontolateral laryngectomy, 1 total laryngectomy 47 Some patients had later a recurrent cancer and underwent a more radical operation and/or received radiation therapy Of these some had unilateral or bilateral neck dissection

As it is not the purpose of the present study to discuss the efficacy of the therapy and operative problems of the larynx cancer, we do not report the treatment of each patient, though it has been carefully analysed and recorded

Biopsy specimens were fixed in 10% formolsaline, embedded in paraffin and then stained with haematoxylin and eosin All histological sections were reviewed by the same pathologist (A Ferlito), who graded the lymphocytic and plasma cell infiltration as minimal, moderate or marked, according to its intensity As to histological grading, squamous cell carcinoma was classified as follows grade I (well differentiated) grade II (moderately well differentiated) and grade III (poorly differentiated), according to the degree of tumour cell differentiation The histologic criteria taken into consideration were keratinization, epithelial pearls mitotic activity, cellular bridges, nuclear-cytoplasmic ratio, anaplastic cells

RESULTS

Before reporting the results, we would like to stress that lymphoid inflammatory infiltrates surrounding tumour necrosis were not taken into consideration, in order to avoid assigning inappropriate significance to the presence of lymphocyte and plasma cell infiltration, which in our study was always evaluated and demonstrated independently from regressive phenomena Polymorphonuclear leukocytes infiltrating the tumour stroma were not considered

Fig 1 shows the survival curves of patients Of 104 patients 58 were alive at 5 years, which means a percentage survival of 55 77% In establishing the survival, deaths for causes other than the malignant disease were included Therefore the curve reflects the absolute survival rate irrespective of the cause of death at 5 years after operation or after diagnosis when no treatment was given

Figs 2A and 2B respectively show the number of patients and relevant survival curves as related to histologic grade of

Figs 5 6 Note the abundant lymphoplasmacellular infiltration around the tumour (H & E ×200)

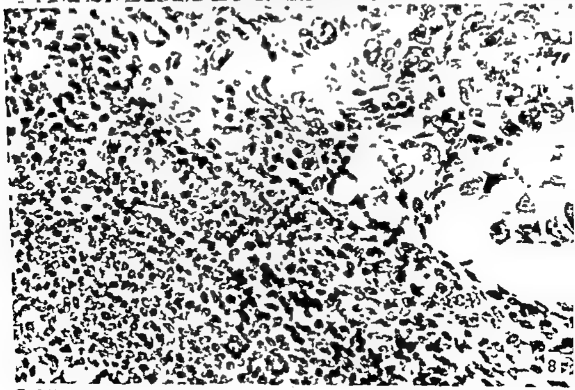

Fig. 7. Higher magnification shows better how the tumour appears to be almost delimited by cellular response (H & E ×300)

Fig. 8. Note cellular infiltration around the tumour (H & E ×500)

cell carcinoma the cellular differentiation of which is marked, is very good, as generally reported in the literature (Kraus & Perez-Mesa, 1966, Biller et al , 1971, van Nostrand & Oloffson, 1972, Ferlito et al , 1975), irrespective of the site of origin of the tumour, whereas in the undifferentiated oat cell carcinoma of the larynx the degree of malignancy is high and there is a tendency to early metastases (Olofsson & van Nostrand, 1972, Ferlito, 1974, Gelot et al , 1975)

That the highly differentiated tumours have, other conditions being equal, a better prognosis than the less differentiated neoplasms, is an established fact The classifications into grades of cellular differentiation for the squamous cell carcinoma of the lung is also advised by the Expert Committee on Lung Cancer formed at the initiative of the World Health Organization (Kreyberg et al , 1967)

Unlike other organs, such as, say, the breast, carcinoma exhibits in the larynx the same pathomorphological pattern throughout the whole tumour A correlation may then be established between the histological type of the tumour and its biological and clinical behaviour This is impossible when a neoplasm is polymorphic and shows solid, glandular and is areas simultaneously

study of peritumoural lymphocyte and na cell infiltration as an index of the host's nune response is another valid criterion in establishing the prognosis

From an accurate examination of our results, it appears that there is a correlation between lymphocytic and plasma cell infiltration in peritumoural and tumoural stroma and the length of survival of cancer patients The cellular stromal reaction reflects the host s immune response against tumour and therefore may be considered as a favourable prognostic sign This confirms the investigations by Bennet et al (1971), but not what affirmed by Underwood (1974), who partly ascribes lymphoid infiltration to the presence of necrotic foci in the tumour rather than to cellular immune reaction by the body

As a matter of fact lymphocyte and plasma cells are conspicously present in some tumours, sometimes with germinal centre formation, irrespective of the presence of necrotic phenomena, as may be noted in all verrucous squamous cell carcinomas of the larynx (Ferlito et al , 1975) in which, besides, necrotic foci are never seen

On the other hand, in spontaneously regressing malignant tumours, immunologic factors identifiable in the presence of immunocompetent cells, such as lymphocytes and plasma cells, may play a role that causes the total regression of the tumour already histologically diagnosed (Boyd, 1966)

Therefore, a causal relationship may be established between abundance of lymphocytes and plasma cells around the tumour and a beneficial prognosis, or defect in cellular immune response and a rapidly fatal course of the malignant disease

In support of the aforesaid, in our series the survival at 5 years rose above 80% for patients with both marked or moderate cellular response within Grade II tumours but dropped to 40% for those with minimal or absent lymphoplasmacellular infiltration For Grade I tumours, the 5 year survival rate is proportional to the degree of histologic host response (minimal 80%, moderate 83 33%, marked 87 50%) In our series, patients with grade III carcinoma of the larynx showed minimal or no cellular response of 20 patients, only 4 survived at 5 years (survival rate 20%, Figs 4 A and 4 B)

These conclusions are in agreement with recent studies on tumour regional lymph nodes and on peripheral blood lymphocyte count, that is to say, on homologous cell groups distinguishable only upon their location As to pathomorphological evaluation of regional lymph nodes it appears clearly that an increase in lymphocyte level in regional lymph nodes is a reliable indicator of the host s cellular immune competence

Any tumour has a more favourable prognosis when regional lymph nodes exhibit a pat

tern of 'lymphocyte predominance' These studies were made on regional lymph nodes of patients with cancer of the breast (Tsakraklides et al, 1974) or of the uterine cervix (Tsakraklides et al, 1973) Malicka (1971) is of the opinion that in laryngeal cancers the presence of sinus histiocytosis in the regional lymph nodes seems to be a favourable prognostic sign Zechner (1975) states that patients with intense stromal reaction and sinus reaction in lymph nodes live 5 years and longer after operative treatment

Investigations have been extended to peripheral lymphocyte count Recently, Papatestas & Kark (1974) carried out a retrospective study of 305 patients with breast carcinoma treated at the Mount Sinai Hospital of New York On the basis of peripheral lymphocyte count made prior to treatment, using the "student's test", it appeared that the survival rate at 5 years was higher for patients with the highest count Therefore, peripheral lymphocyte counts may well reflect the host's immune response to tumour growth and could serve as a prognostic indicator (Riesco, 1970, Papatestas & Kark, 1974)

Stjernsward et al (1972) observed lymphocytopenia in peripheral blood following radiotherapy, with a significant shift in the proportion of T and B lymphocytes, with an absolute and relative decrease of T cells

All these investigations seem to prove that lymphocytes, whether in the tumour and peritumoural stroma or in lymph nodes draining tumour area, or in circulating blood play an active role in the immunologic surveillance Is the lymphocyte a 'killer' of cancer cells? Animal studies have confirmed that in immune animals T-lymphocytes recognize and destroy the tumour cells thus causing spontaneous regression of the lesion These lymphocytes behave as 'killers' and represent the immunologic response against the tumour Marx (1975) states that the thymus dependent lymphocyte is a killer a helper and a suppressor

Consequently it appears that the lymphocyte is the basic element of the immune mechanism, when it comes into contact with tumour antigens it changes into a pyroninophilic blast cell which in turn is capable of producing immune lymphocytes According to Mackler (1971), these immune lymphocytes when coming into contact with tumour cells release lymphocytotoxin which has a specific cytotoxic effect on the tumour cell, the outer membrane of which is destroyed Around the tumour an accumulation of immune lymphocytes is formed, they would produce the Transfer Factor (TF), that is, a factor capable of changing non-immune lymphocytes in active, specific immune cells capable of destroying the tumour cells

Immunologic surveillance is of basic importance in the study of tumours Whenever there is a deficit in cellular immunity, the body's defensive reaction against cancer is impaired Immunologic surveillance is a defensive and protective action The thymus produces small lymphocytes—T-lymphocytes, so as to distinguish them from B lymphocytes produced by lymph nodes— which are an indicator of the host's cellular immune competence When the immunity surveillance is somewhat impaired—as it happens in patients with genetic immune deficiency, or in elderly people or again in patients treated with immunosuppressive agents (Cobau et al, 1973, Penn & Starzl, 1973, Marshall, 1974, Penn, 1974) or chemotherapy or radiation—the development is more likely of one or more malignant neoplasms, the prognosis of which will be almost always poor For this purpose, the frequency must be mentioned of tumour occurrence in kidney transplant recipients treated with immunosuppressive drugs (Penn, 1970)

Gatti & Good (1971) state that patients with a defective cellular immunity are 10 000 times more likely to develop a neoplasm than the general population with normal immune response potential

The present trends in cancer investigation must consider the immunologic potential of

the cancer patient The most difficult question to be answered is whether the immune deficiency is the cause or the effect of cancer However, it is a well-known fact that according to experimental data, a deficit in cellular immune response is always a factor favouring tumour growth Mice subjected to thymectomy at birth are most likely to develop tumours induced by different carcinogens In rats subjected to tumour transplantation, the injection of lymphocytes of rat produced a regression of the neoplasm proportional to the number of lymphocytes injected (Delorme & Alexander, 1964) Ridley (1971) observed that transplantation in the brain of rats of neoplasms of hamsters induced by polyoma virus caused a rapid growth of the tumour in the animals previously treated with antilymphocytic serum In humans, lymphocytopenia has been found in association with tumour (Zacharski & Linman, 1971)

Trials have been made to cure cancer with thymosin, a hormone extracted from thymus and capable of activating the body s cellular immune response (Marx 1975)

As to the present investigation it can be said that the presence of cell mediated immune reaction in the stroma is an important ment in establishing the prognosis which is more favourable the more marked is the mphocyte and plasma cell infiltration Figs 5 6 7 and 8 show a marked infiltration found in a case of squamous cell carcinoma of our series

Tumour cell differentiation and lymphocyte and plasma cell infiltration must therefore be considered as valuable parameters in establishing the prognosis of the larynx cancer Lymphocytic and plasma cell infiltration must be regarded as the host s response to tumour growth and appear to be the less evident the more undifferentiated the neoplasm

Therefore a relationship between histologic grade and lymphocytic and plasma cell infiltration seems to exist however this relationship is not always present and is possibly mediated by other factors It is worth emphasizing that the length of survival is mainly a function of histologic grade Cellular response is another important factor, though secondary to cellular differentiation of the tumour Within the single classes of histologic grade, the survival rate increases with the increasing intensity of lymphocyte and plasma cell infiltration

RESUMÉ

Une série de 104 cas de carcinome squameux du larynx a été etudiee quant a l interaction entre tumeur et organisme Le pronostic a ete evalue selon la differentiation histologique de la tumeur et la reponse immunitaire jugee par la presence d une infiltration lymphocytaire dans le tissue conjonctif tumoral La differentiation histologique et la reaction immunitaire semblent etre en relation avec la survivance à 5 ans La reponse immunitaire semble toutefois avoir une valeur pronostique favorable seulement dans les tumeurs differenciees puisque dans notre serie tous les cancers indifferencies montraient une infiltration stromale minime ou absente Le taux de survivance augmente avec la croissante intensite de la reponse cellulaire dans une même classe histologique Les petits lymphocytes sont les elements fondamentaux de la reponse immunitaire cellulaire Après contact avec un antigene tumoral ils deviennent des immunoblastes et produiraient de lymphocytes avec memoire immunologique qui reconnaitraient et detruiraient les cellules tumorales

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VOLUNTARY, NON VISUAL CONTROL OF THE HUMAN VESTIBULO OCULAR REFLEX

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Abstract Voluntary control of the human vestibulo-ocular reflex with and without visual targets was investigated Subjects were rotated sinusoidally from 0 1 to 1 0 Hz using d c electro oculography to record eye position The ratio of eye to head movement or gain of the vestibulo-ocular reflex was measured When subjects were rotated in the dark at 0 3 Hz whilst performing mental arithmetic the gain was 0 65 When subjects were asked to fixate imaginary targets in the dark that were stationary in space the gain rose to 0 95 When they imagined targets rotating with them on the chair the gain dropped to 0 35 Our results indicate that the ability to modulate the gain of the vestibulo-ocular reflex does not depend entirely on the smooth pursuit system Higher centers must modulate eye velocity so that it is appropriate to the subject s choice of a frame of reference whether or not vision is available

The vestibulo ocular reflex developed in evolution to allow animals and man to keep a stable image on the retina Head movements in one direction are countered by eye movements of equal magnitude in the opposite direction so that objects in the visual field remain clear The pathways of this reflex—from the vestibular nuclei through the reticular formation and the medial longitudinal fasciculus to the extraocular motor nuclei and muscles—have been known for many years and vestibular physiologists have used it as a qualitative and quantitative measure of vestibular function Inspection of the nystagmoid patterns observed with caloric testing and in post rotatory nystagmus are two of the more common tests used to evaluate the integrity of this reflex

It seems to us, however, that more might be learned if we tested this system by seeing if it can do what nature designed it to do make slow-phase eye velocity equal (but opposite) to head velocity The ratio between these two movements is called the gain of the vestibulo ocular reflex Normally the gain is equal to one as can easily be seen in most of our daily visual tasks, if it were not, vision would be blurred each time we moved our heads Measuring the gain of the reflex might be a useful clinical tool in investigations of the vestibular and oculomotor functions of patients, especially, perhaps, in measuring the integrity of the central pathways To use such a test it is first necessary to describe the situation in normal subjects

Previous studies (e g , Meiry, 1971) indicate that when subjects are rotated sinusoidally in the dark in the frequency range around 0 1 Hz, the gain of their vestibulo-ocular reflex is only 0 4 to 0 6, but rises to about 1 0 at high frequencies (Benson, 1970) The gain also depends on mental alertness and it has become customary in testing to have subjects do mental arithmetic (e g Collins, 1962) Since the gain seems to depend on mental state, we

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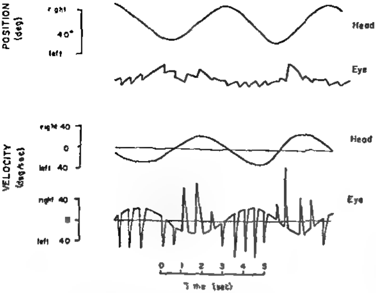

Fig 1 (A) Typical tracing of head and eye position and velocity while subject was performing mental arithmetic in the dark The frequency is 0 2 Hz

wondered to what extent subjects could voluntarily control the gain of their reflex in response to various instructions In short, it was necessary to investigate what factors could influence the gain and by how much As it turns out, subjects can voluntarily change the gain of their reflex, in the dark, from 0 2 to 1 0

The question also arises if the gain at low frequencies is 0 6 in the dark with mental thmetic, but can become 1 0 or 0 0 with an opriately moving visual stimulus, how s the central nervous system achieve this? e obvious answer is that the smooth pursuit system is responsible for augmenting or suppressing vestibular eye movements However, the fact that subjects can alter the gain without vision raises doubts about this simple explanation It is of interest to investigate in what ways the gain changes seen in this study cannot be accounted for by the smooth pursuit system

METHODS

The subjects were 12 men and 1 woman between the ages of 15 and 48 years They sat normally in a Barany chair and were rotated horizontally by hand, either with short, high-velocity transients up to 130 deg/sec or sinusoidally over the frequency range 0 1–1 0 Hz at amplitudes not exceeding ±40 deg The head was stabilized by a head rest and, when necessary, a bite bar Chair position (and thus, head position) was recorded by a potentiometer attached to its base Horizontal eye position was recorded by d c

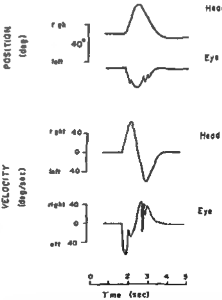

Fig 1 (B) Typical tracing of head and eye position and velocity with a high frequency velocity transient while subject was performing mental arithmetic in the dark Peak head velocity is 64 deg/sec peak eye velocity is 60 deg/sec

Table I *The gains of the vestibulo ocular reflex at different frequencies under different experimental conditions*

The gains are the mean of the population studied The standard deviations show the distribution of individual means Typical intrasubject standard deviations are shown in Fig 2 and discussed in the text Stationary refers to a real or imagined target fixed to the wall of the room Moving refers to a similar target rotating on the chair with the subject

Situation	0 3 Hz	0 8 Hz	HF
1 Visible stationary target	1 0	1 0	1 0
2 Imaginary stationary target	0 94±0 04	0 96+0 04	0 95±0 07
3 Dark mental arithmetic	0 65±0 06	0 83±0 04	0 97±0 03
4 Visible moving target	0 05	0 10	0 60±0 08
5 *Imaginary moving target*	*0 32±0 07*	*0 44±0 04*	*0 64±0 14*
6 After image stationary	0 94±0 07	0 96±0 02	
7 After image moving	0 33+0 13	0 44+0 08	
8 9 Ganzfeld lens mental arithmetic			
light	0 71±0 06		
dark	0 69±0 07		

electro-oculography using bi temporal electrodes The frequency response of the amplifier was 0 to 100 Hz Eye position and chair position signals were stored on magnetic tape and reproduced with equipment whose bandwidth was well in excess of this, at which time they were also electrically differentiated with time constants of 0 1 and 0 05 sec respectively, to obtain eye and head velocity The gain of the vestibulo-ocular reflex was measured by dividing peak eye velocity by peak head velocity for 8–10 half cycles for each experimental run

To minimize drift in the sensitivity of the EOG, experiments were carried out in a room illuminated with dim, red light All subjects were dark adapted until successive recalibrations showed that the corneoretinal potential had stabilized (usually about 15 min) The EOG was recalibrated by having the subject look between two fixation points 20 deg left and right of the midline, before and after each part of the experiment (thus about every 2–3 min) Base line drift never exceeded 2–3 deg over this period and EOG sensitivity changes were less than 6% If the sensitivity between calibrations changed significantly, the mean calibration was used in analyzing the intervening data The overall accuracy of the eye recordings was estimated to be better than ±2 deg Subjects were instructed to keep their eyes open throughout the experiment

In some experiments after-images in the form of a cross centered on the fovea, were given with a flash gun In others, a Ganzfeld or formless, illuminated visual field, was created by using room illumination with a translucent scleral contact lens in one eye, the other eye was covered in such a way that the lids of both eyes could remain open normally Because of sensitivity drift, the EOG was recalibrated frequently during this part of the experiment In still other experiments a small, dim, red fixation lamp was used, either fixed to the wall of the room, or attached to the Barany chair, in front of the subject

RESULTS

The first project was to reinvestigate the way in which the gain depended on frequency of sinusoidal rotation during the control situation in which the subjects attention was occupied by mental arithmetic

Fig 1A shows tracings typical of head and eye position and velocity The gain of the vestibulo ocular reflex is the ratio of peak slow phase eye velocity to head velocity. The

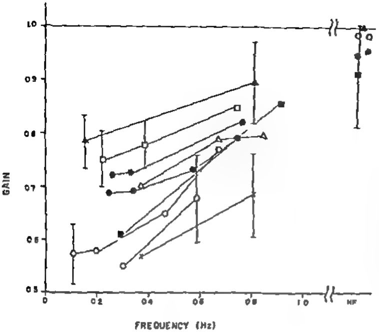

Fig 2 The gain of the vestibulo-ocular reflex of nine different subjects during sinusoidal rotation in the dark, while performing mental arithmetic, plotted as a function of frequency Each data point is the mean of 8 to 10 individual measurements A few typical standard deviations for individual subjects are shown by vertical bars *HF* indicates the high frequency range represented by the transient experiments illustrated in Fig 1B

in which the gain of 9 subjects depended on frequency is shown in Fig 2 and Table I (row 3) For all subjects it rose with increasing frequency As shown in Fig 2, typical standard deviations for any one subject were ±0 07 This did not depend on the particular task This number did increase at the higher frequencies The mean gain for the group rose m 0 65 at 0 3 Hz to 0 83 at 0 8 Hz However, there was considerable variation among individuals At 0 3 Hz, for example the range individual means was 0 57 to 0 76 This variability became less at higher frequencies where all gains tended toward 1 0

It was difficult to rotate the chair at frequencies above 1 Hz, yet normal head movements must have frequency components well above that To obtain a rough estimate of the gain at higher frequencies *sharp transient* chair movements of sudden onset were used, as shown in Fig 1B Generally the chair could be brought up to some velocity such as 60 deg/sec (as shown) in about 300 msec This was followed by a rather short period of roughly constant velocity before the chair was decelerated The gain of the vestibulo ocular reflex was taken as the ratio of peak slow phase eye to chair velocity and was considered in this study to represent the behavior of the gain in the frequency range of natural head movements somewhere above 1 Hz During mental arithmetic this high frequency gain (HF, Fig 2) was 0 97 for the group

It is assumed that rotation in the dark with mental arithmetic prevents the subjects from attending to any oculomotor task We next asked them to look at imaginary targets in the dark As controls, subjects were first asked to fixate target lights in front of them that were either fixed to the wall or to the Barany chair Subsequently, the target lights were turned out and the subjects were instructed to go on looking at one or the other as though they were still visible When subjects fixed a visible target light on the wall, eye movements were always equal and opposite to head movements at all frequencies (Fig 3, open squares) so the gain was essentially 1 0 Similarly, when subjects were asked to look at a target light that rotated with them, their eyes moved very little in their heads so the gain was nearly zero (Fig 3 open circles, Table I row 4) However as the frequency of sinusoidal rotation was increased to 0 8 Hz subjects were less able to suppress

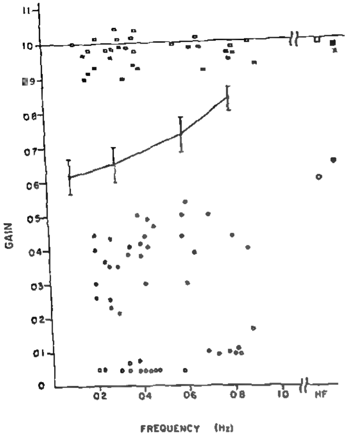

Fig 3 The gain of the vestibulo-ocular reflex of all twelve subjects under varying conditions plotted as a function of frequency of rotation Each data point is the mean of 8 to 10 measurements each subject represents from one to four data points in any one experiment For simplicity no distinction is made between individual subjects □ Subjects fixating a target light stationary in space ○ subjects fixating a target light rotating with them on the chair ■ subjects looking at an imaginary target fixed in space in the dark ● subjects looking at imaginary targets moving with them on the chair in the dark × s indicate gains during rotations in the dark with mental arithmetic (this is the data from Fig 2 averaged over the population) HF indicates the high frequency range of velocity transient experiments the symbols represent the mean values of the whole group for each experiment

their vestibulo-ocular reflex and the gain rose slightly to about 0 10 At high frequencies, the gain rose still further to 0 60 although there was much variation in the mean gain between individuals, ranging from 0 46 to 0 67 (a typical record of this transient, high frequency experiment is shown in Fig 5)

The target lamps were then turned off and the subjects were asked to both imagine and continue tracking them in complete darkness Subjects were also rotated sinusoidally in complete darkness without fixation lamps and asked, at unexpected times, to switch from one imaginary target to the other (on the wall or on the chair) and to continue to track it There was no difference in the results between these two procedures Typical results are shown in Fig 4 There was always a significant change in the amplitude of eye displacement and velocity when attempted fixation was shifted from one imaginary target to the other As shown by Fig 4, subjects could augment or depress their gain for long periods of time not simply in a momentary or transient fashion To assist them, they were constantly reminded by the

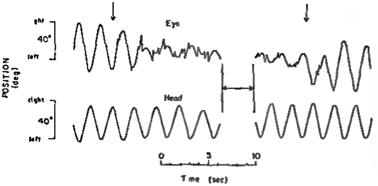

Fig 4 Record of one subject demonstrating voluntary change of gain while rotated in complete darkness at 0 4 Hz The subject was initially looking at an imaginary target fixed in space the first arrow indicates the point at which he was asked to imagine a target rotating with him the second arrow indicates the point at which he was asked to resume imagining a target fixed in space The time lag between the two vertical lines in the center of the record was 33 sec

experimenter of which imaginary target they should attempt to fixate The gain under these conditions of imaginary target fixation showed a large standard deviation The mean gain for the group for fixation of an imaginary target stationary in space was about 0 95 and also independent of frequency (Fig 3, filled squares, Table I, row 2)

Intersubject variability and standard deviation of individual gains was especially large for fixation of an imaginary target moving with the subject in the dark (Fig 3, filled circles, Table I, row 5) Nevertheless, mean values of the gain over the population were depressed to 0 32 at 0 3 Hz and 0 44 at 0 8 Hz At the h frequencies of velocity transients, the n gain was 0 64±0 14 (S D), and in vidual mean values ranged from 0 41 to 0 81

typical record is shown in Fig 5 It should be noted that only the velocity of the first slow phase was used in analyzing records such as those in Fig 5 Whether a target was visible or not, quick phases kept mean eye position near the real or imagined target, and slow phase eye velocity quickly slowed down But the first quick phase usually occurred within 100 msec of the start of rotation (as in Fig 5) so the velocity of the first slow phase is presumably not affected by the pursuit system which is well known to have a reaction time of about 130 msec (Robinson, 1965) At any rate, Fig 3 shows that at lower frequencies of rotation, each subject could raise or lower his gain so significantly above or below the value for mental arithmetic by the effort to fixate imaginary targets, that there was no overlap of the data points for the different situations over the population Some subjects, in individual trials (Fig 3) could switch their gain from 0 2 to 1 0 by changing their imagined frame of reference

It has been reported by Yasui (1974) that the gain of the reflex improves when subjects *look at after-images Consequently, our subjects* were given an after image and instructed to keep it either fixed in space or moving with them as they were rotated The results (Table I, rows 6, 7) showed little difference compared with their efforts to perform the same task with imaginary targets (Table I, rows 2, 5)

Both the attempts to fixate an imaginary target or an after-image decreased the number *of quick phases seen in the nystagmoid pattern* of eye movements (e g , Fig 1 A compared with Fig 4 when the subject fixed an imaginary stationary target) Using mental arithmetic at 0 3 Hz with an excursion of about ±20 deg as a standard, the average number of quick phases per cycle was calculated for 10 cycles for each subject The mean value for 12 subjects was 4 4 (range from 2 2 to 7 6) In each case except one, the number of quick phases dropped to an average of 2 1 (range from 0 6 to 4 4) when subjects attempted to fixate a stationary, imaginary target in the dark at 0 3 Hz, and to 2 6 (range 0 5 to 4 0) with attempts to fixate an imaginary target moving with the chair at 0 3 Hz With attempts to fixate an after image

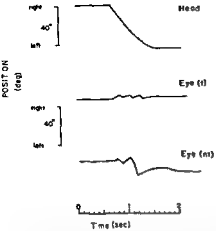

Fig 5 Typical tracing of head and eye position with a high frequency velocity transient while the subject was either looking at a visible target (Eye *(t)*) or at an imaginary target in the dark (Eye *(nt)*) that moved with him as he was rotated In both cases the initial gain was 0 6 and the first quick phase occurred within 100–200 msec

and keep it stationary in space, there were almost no quick phases (0 5 per cycle at 0 3 Hz) Thus, although after images did not help in voluntary control of gain, they did help to suppress quick phases

Although the action of the visual system through the smooth pursuit system could account for the rise (to 1 0) or the drop (to 0 05) in gain when target lamps were visible we wondered if light alone, devoid of visual structure could somehow activate the nervous system and change the gain Consequently, a translucent contact lens was used to create a Ganzfeld and subjects were rotated while performing mental arithmetic They showed no difference in gain between light and dark at any frequency (see Table I, rows 8, 9 for 0 3 Hz) The gains of individual subjects did not differ by more than ±0 03 between light and dark at any one frequency Because of the slight stress created by the lens and patch, the gain in the light was compared to that taken in the dark immediately afterward but, as it turned out, the two gains for mental arithmetic in the dark (Table I, rows 3 and 9) were not very different in spite of the differences in experimental conditions

DISCUSSION

It seems reasonable that the per-rotatory gain of the vestibulo-ocular reflex should be a valuable measurement in neurological examinations simply because it is a quantitative measure of this system's natural function It has already been noted (Zee et al, 1974) that downbeat nystagmus, associated with possible vestibulo-cerebellum disorders arising from the Arnold Chiari malformation, is characterized by abnormally large gains (greater than 4 0) in the vertical reflex As the test is applied in a greater variety of pathological situations, patterns will probably emerge which will have diagnostic value

One thing is clear from this study any instruction to the patient is better than none at all Without instruction, a subject or patient could unconsciously adopt a strategy of paying more or less "attention" to his stationary surroundings that could change the gain from 0 60 to 1 0 In some situations, usually aerospace medicine, a subject is rotated in an enclosed cab In that case, by "paying attention" to an earth fixed or cab-fixed frame of reference, the gain could vary from 0 2 to 1 0 To avoid this possible five fold variation, the subject should always be given a specific instruction What instruction and what type of rotation might be preferable for clinical testing?

It is not surprising that results are most reproduceable at high frequencies This is because the major use of this reflex in daily life is to stabilize images during natural head movements in which the stimulus is usually brief (e g , 0 5 sec) and thus characterized by high frequency spectral components A gain of 1 0 is always needed in this case and the central nervous system, probably by some form of parametric adaptation (Robinson, 1975), makes sure that the gain stays at 1 0 Voluntary efforts to interfere with the high-frequency gain are not very successful Unfortunately, this test is technically difficult because good head stabilization is needed

(e g a bite bar) to make sure that rotating the chair actually does rotate the head

At lower frequencies, probably the simplest test is to have the subject look at a stationary target and imagine and continue to look at it after it goes out This instruction is very simple to understand and execute Its advantages are, fixating the lamp is a good calibration of the head and eye position monitors since in all but severe pathological cases, the gain will be exactly 1 0, quick phases are largely suppressed making the slow phase velocity easier to obtain from the records, and finally, one is giving the subject a natural task to perform in which he is encouraged to allow his reflex to do the best it can, a situation that leads to less intersubject variability as can be seen by comparing Fig 3, filled squares with Fig 2.

However, if one has gone to the trouble of measuring head and eye velocity, it would be a pity not to do all four tests (Table I, rows 1, 2, 4 and 5) because they also test vestibular visual interaction and the patient s ability to use visual and nonvisual central mechanisms to modulate the reflex gain There is good reason to suppose (Lisberger & Fuchs, 1974, Westheimer & Blair, 1973) that, in ates, the cerebellum is an important icture for visual vestibular interaction 'S Zee (unpublished observations in this laboratory) has already observed that some patients with familial cerebellar degeneration have deficient smooth pursuit systems and cannot suppress their vestibulo-ocular reflex to allow them to fixate a target lamp moving with them on the chair Consequently, we are reluctant to recommend just one specific instruction to the subject since the tests with all four instructions are as simple to do as with one and provide a good deal more information of diagnostic value about the functioning of the central vestibular pathways

One should note in passing that the number of quick phases per cycle is highly idiosynchratic and a remarkably poor indicator of whether the subject's reflex is normal There was a 3 to 1 spread in this measure over the group studied in the mental arithmetic situation The spread was 8 to 1 in other situations and, even without after-images, their number dropped by a factor of two when subjects were asked to fixate imaginary targets It seems to us that it is rather difficult to conclude anything about slow phase gain by counting quick phases

From a theoretical standpoint, the interesting aspect of these results is their bearing on the question of how the pursuit system interacts with the vestibulo-ocular reflex Fairly accurate theoretical models exist for these systems when they act alone When both the observer and the target are in motion at the same time, the question arises as to whether the results can be explained by assuming that the two systems continue to operate normally and interact only by linearly combining their signals at the final common path While no one has defended this rather simple idea, the present results indicate conclusively that this cannot be the case

There are two results in this study that cannot be explained by the action of a smooth pursuit system The first is that eye velocity during rotation in the dark can be altered by fixating an imaginary target It could be argued that the pursuit system was still responsible but was being driven by nonvisual imagery rather than real visual motion However, it's well known that one cannot make smooth pursuit movements without a moving visual stimulus There are a few situations where nonvisual smooth tracking occurs one can make smooth eye movements behind closed lids, one can smoothly track one s hand in the dark (Steinbach 1969) smooth movements can be made with the aid of after-images (Kommerell & Klein, 1971) (it is significant in our results that after images did not assist in nonvisual modulation of vestibularly induced eye velocity), a few people can make nonvisual smooth movements either naturally or by training

None of these situations alters the fact that

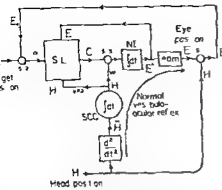

Fig. 6. The schematic organization of the vestibulo-ocular reflex and visually guided eye movements. The semicircular canals (*SCC*), neural integrator (*NI*) and extraocular muscles and globe (*eom*) constitute the normal vestibulo-ocular reflex. Retinal input (*e*) comes from comparing target position (*T*) with eye position (E_s) in space. The main idea is that a part of the central nervous system involved in spatial localization (*SL*) receives visual information (*e*), an efference copy of eye position (*E*) and vestibular information ($\dot{H}$) and combines them with an internal choice of a stationary or moving frame of reference and issues an oculomotor velocity command ($\dot{C}$) which modifies eye velocity and the apparent gain of the vestibulo-ocular reflex. Details are given in the text.

most subjects are totally unable to make smooth movements in pursuit of an imaginary target in the dark with eyes open. Even when tracking a visible target moving at constant velocity, tracking becomes totally saccadic in about 0.3–0.5 sec after the target disappears. Yet in this study, all the subjects could make large changes in eye velocity with no visual image at all. With a swing of ±40 deg at 0.3 Hz the peak head velocity is 75 deg/sec. When a subject changes his reflex gain from 0.65 to 1.0 or 0.2 by mental imagery in the dark, he changes his peak eye velocity by 26 or 34 deg/sec *respectively*! Such velocities cannot possibly be produced by the smooth pursuit system in the dark when the head is still.

The second result can be seen in the transient experiments in which the subjects are asked to look at a real or imaginary target moving with them. If they are rotated by a transient that reached, say, 100 deg/sec and they had dropped their gain from 1.0 to about 0.62, their eye velocity was depressed from 100 deg/sec to 62 deg/sec (a change of 38 deg/sec) and this decrease was apparent during the first 100 msec of the rotation. The latency of the pursuit system is about 0.13 sec (Robinson 1965) so that even in the case where the target was visible, the pursuit system did not have time to create the velocity decrease. Clearly the gain had been preset before the rotation began by the instruction to the subject.

Evidently then, the gain of the vestibulo-ocular reflex is under the control of a central mechanism concerned with spatial localization, that is, in manipulating the central connections of the reflex to make it suitable for looking at objects that are either stationary in space or fixed relative to the head. (These are the two most common situations, the former for looking at the normal visual surround, the latter, for a manipulative animal, for looking at objects being carried in the hand.) This mechanism does not require vision but only the percept of the relative motion between self and the environment of interest. Vision can obviously assist this mechanism and greatly improve its performance but the important point is that a large part of the eye movement control that makes the gain tend to 1.0 or 0.0 does not depend on vision and is not produced by the so-called pursuit system.

These ideas are illustrated in Fig. 6. Summing junction SJ1 simply indicates that eye position in space, E_s, is the sum of head position in space, H, and eye position in the head, E. SJ2 indicates that retinal error, e, is the difference between target position in space, T, and eye position in space, E_s. Head acceleration, $\ddot{H}$ (the second derivative of H), stimulates the semicircular canals. Because cupula dynamics are heavily overdamped, the signal entering the brain stem on primary vestibular afferents is *approximately proportional to head velocity*, $\dot{H}$, *over the* bandwidth of natural head movements (Fernandez & Goldberg 1971, Melvill Jones & Milsum 1971). It is *now well* established

(Carpenter, 1972, Skavenski & Robinson, 1973, Shinoda & Yoshida, 1974) that this signal is integrated once more by a neural net work (NI) in the brain stem to produce a neural eye position command, E', that creates, through the extraocular muscles, an eye movement that is equal and opposite to head position thus providing the basic vestibulo ocular reflex

We imagine that some form of velocity cor rection signal, $\dot{C}$, is added to the vestibulo-ocular reflex (at SJ3) so as to adjust the gain of the reflex appropriately, raising it to 1 0 or dropping it to 0 0, depending on what is needed The question raised by this study is how this signal is created by the brain Fig 6 shows the neural substrate for this, segregated into a compartment marked SL whose function, in a broad sense, is that of spatial localization or calculating the relative motion between the observer and some external frame of reference

This mechanism obviously uses vision when it is available A subdivision of SL which converted retinal error, e or better, retinal slip, $\dot{e}$, into an appropriate $\dot{C}$ when the head was still would simply be called the ursuit system

Yasui (1974) has suggested that efference copy, E', is also important in the pursuit ystem If the head is still and one adds e to E', one recreates, in SL, a neural signal (possibly the percept) of target position (or velocity) relative to the head (not just relative to the eye) This percept could then act to drive the eye through $\dot{C}$, in smooth pursuit and could explain how it is that the eye can pursue an after image even though there is no retinal slip which is classically thought to be the *sine qua non* of smooth pursuit movements There is mounting evidence (see Yasui (1974) for a review) that efference copy plays an important role in oculomotor control as well as spatial localization and E' must be considered as one of the pieces of information that SL might use to produce smooth pursuit movements

However, the results of this study indicate that whether e or both e and E' are used to generate smooth pursuit movements, that mechanism only partly accounts for the modulation of vestibular eye movements observed It is suggested in Fig 6 that there is a direct, more or less fixed, vestibulo-ocular reflex, VP1, that works independently of SL This could be the pathway that creates the nystagmus seen during mental arithmetic in the dark ($\dot{C}=0$) Modulation of the reflex suggests that semicircular canal afferents ($\dot{H}$) also project to SL (path VP2) for use in the total calculation of the appropriate $\dot{C}$ It is this path (from VP2, through SL to $\dot{C}$) that appears to be modulated nonvisually by the subjects' efforts to stabilize their eyes in a fixed or moving frame of reference It seems necessary to suppose that the subject calculates his head position (or velocity) from $\dot{H}'$, makes an internal choice of a reference frame that is moving with him (no relative motion) or is stationary (a relative motion of $-\dot{H}'$) and then calculates the correct proportion of the $\dot{H}'$ signal to pass out in the form of $\dot{C}$ to make the eyes move in the desired way

How all this is done is unknown and there are many details to be worked out concerning how the central nervous system mixes visual and vestibular information to produce oculomotor behavior The most we can say here is that the raw materials needed are retinal error (when available), an efference copy of eye position and the semicircular canal afferents and this information is manipulated by internal decisions so that the final vestibulo-ocular reflex is suitable to one or another frame of reference This control of eye movements is not done totally by the smooth pursuit system

It has already been mentioned that the vestibulocerebellum (especially the floccular nodular lobes) may be involved in this phenomenon Westheimer & Blair (1973) have shown that smooth pursuit movements in the monkey are abolished by cerebellectomy

Takemori & Cohen (1974) have shown that floccular lesions in monkeys interfere with their ability to use vision to suppress vestibular nystagmus Lisberger & Fuchs (1974) have shown that Purkinje cells in the vestibulocerebellum modulate their firing rate in phase with the canal afferents when monkeys are rotated sinusoidally in the dark When they suppress their reflexes by fixating target lamps rotating with them, the modulation increases five fold, an activity that would decrease the gain of the vestibulo-ocular reflex because of Purkinje cell inhibition of second order vestibular neurons As mentioned, D S Zee (unpublished observations in this laboratory) has studied patients with presumed cerebellar lesions who cannot suppress their vestibulo ocular reflex by trying to look at targets rotating with them

These examples of visual vestibular interaction in the cerebellum are only fragments of a puzzle so far but they constitute mounting evidence that, in man, the vestibulocerebellum may be heavily involved in the visual and perhaps nonvisual, modulation of the vestibulo-ocular reflex seen in this study

ZUSAMMENFASSUNG

Die willkurliche Kontrolle des vestibulo-okularen Reflexes beim Menschen wurde untersucht mit und ohne Sehziele Die Versuchspersonen wurden sinusförmig bei Frequenzen zwischen 0 1 und 1 0 Hz gedreht und die Augenposition wurde okulographisch registriert Das Verhaltnis von Augen und Kopfbewegung der Verstarkungsfaktor des vestibulo-okularen Reflexes wurde gemessen Wenn die Versuchspersonen im Dunkeln mit 0 3 Hz gedreht wurden und dabei Kopfrechenaufgaben durchfuhrten war der Verstarkungsfaktor 0 65 Wenn die Versuchspersonen aufgefordert wurden sich raumlich stationäre Sehziele im Dunkeln vorzustellen und diese zu fixieren erhohte sich der Verstarkungsfaktor auf 0 95 Wenn sie sich mitbewegte Sehziele vorstellten erniedrigte er sich auf 0 35 Unsere Ergebnisse zeigen daß die Fahigkeit den Verstarkungsfaktor des vestibulo-okulären Reflexes zu verandern nicht allein vom Augenfolgesystem abhangt Hohere Zentren mussen die Geschwindigkeit der Augenbewegung beeinflussen so daß sie für den von der Versuchsperson gewahlten Bezugsrahmen geeignet ist gleichgultig ob sie wirklich sehen oder nicht

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THE EFFECT OF EYE CLOSURE UPON THE PATHOLOGICAL VESTIBULAR SPONTANEOUS NYSTAGMUS

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Abstract In the course of one year (September 1973 to September 1974) a total of 784 patients were examined with nystagmography We were dealing with patients who consulted us for reasons of equilibrium disturbances sensori neural hearing loss and/or tinnitus In 200 patients we observed a definite vestibular spontaneous nystagmus (SN) in supine position Ocular pendular nystagmus was eliminated On the basis of these 200 electronystagmographic recordings the effect of eye closure upon the pathological spontaneous nystagmus was analysed According to the statistical mean value spontaneous nystagmus with open eyes in darkness was stimulated by eye closure Further evaluation revealed however that 24% of our patients showed spontaneous nystagmus exclusively with open eyes in darkness 30% only with eyes Nevertheless the occurrence of spontaneous nystagmus with either open or closed eyes in darkness in both conditions does not permit topodiagnostic conclusions The possibility of nystagmus inhibition by eye closure must be considered when the case history indicates a vestibular disturbance but a spontaneous nystagmus with closed eyes is absent in the electronystagmography The clinical observations in one patient are described in detail for example To exclude errors one should search for a spontaneous nystagmus following the necessary examination behind Frenzel s glasses—in case of nystagmography—by closed as well as by opened eyes in darkness

The vestibular spontaneous nystagmus is inhibited by visual fixation and low vigilance level, activated however by an increase in vigilance, elimination of visual fixation and according to several authors also by eye closure (Rossberg, 1954 Mahoney et al 1957 Megighian & Waldecker 1961 Naito et al 1963)

However, in the course of the last few years we have repeatedly observed patients in whom the nystagmus was essentially inhibited, or even completely eliminated by eye closure despite controlled mental activity One of the most extreme cases is briefly described here

In case of a 32 year-old hitherto healthy man the tentative diagnosis of an isolated peripheral vestibular loss was established To our surprise the intensive nystagmus seen with Frenzel s glasses could not be demonstrated via electronystagmography with closed eyes despite performance of arithmetical problems Recording with open eyes in darkness however disclosed a nystagmus with large amplitude and high frequency (see Fig 1) Our tentative diagnosis was confirmed by additional investigations

Considering these observations we thought it useful to do systematic investigations in a larger group of patients on the reaction of the vestibular spontaneous nystagmus with open eyes in daylight with open eyes in darkness and with closed eyes The following questions should be cleared up

1 Is it a general rule that a pathological vestibular spontaneous nystagmus is activated by eye closure i e eludes us least frequently?

2 How often is a vestibular spontaneous nystagmus—as in the aforesaid example of an isolated vestibular loss—completely eliminated by eye closure?

3 How is the intensity of vestibular spon

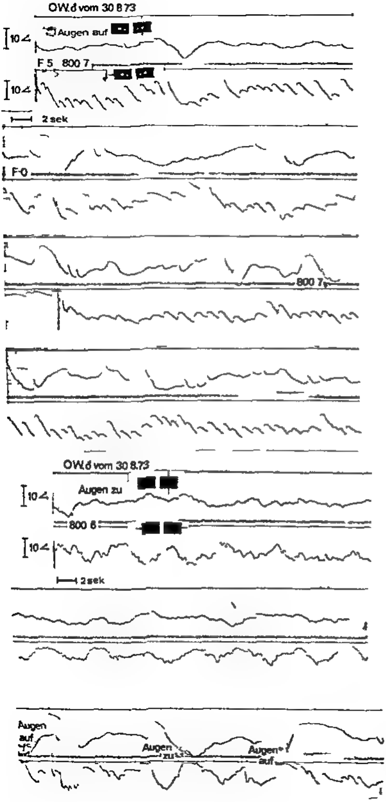

Fig. 1. Complete suppression of an intense vestibular spontaneous nystagmus by open eyes in darkness with lid closure. The figures 800-7 and 800-6 represent the arithmetical problems performed (series subtraction). *Augen auf*: eyes open. *Augen zu*: eyes closed.

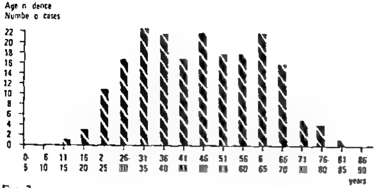

Fig 2

taneous nystagmus with open eyes in darkness and with closed eyes?

4 Is it possible to draw topodiagnostic conclusions from the behaviour of spontaneous nystagmus with open eyes in daylight open eyes in darkness and closed eyes?

METHODS

In the course of one year (September 1973 to September 1974) a total of 784 patients were investigated once or several times The clinical examination consisted both of the search for spontaneous positional or positioning nystagmus by means of Frenzel s glasses and he tests according to Romberg and Unterberger Subsequently prior to the caloric vestibular examination in supine position the eye movements were recorded for 60 sec by electronystagmography with visual fixation as well as with closed and open eyes in darkness We were dealing with patients admitted to our clinic for suffering from vertigo symptoms disturbances in equilibrium sensorineural hearing loss and/or tinnitus Horizontal and vertical eye movements were recorded with a Mingograph (Elema Schonander) at a time constant of 2 5 sec Referring to Henriksson et al (1972) we chose a magnification of a visual angle of 10 degrees corresponding to an amplitude of 15 mm Spontaneous nystagmus intensity was determined on the basis of (i) the sum of the slow nystagmus phases/60 sec in angle degree (ii) beats/60 sec

In this investigation we were concerned with the comparison of recordings and therefore in our opinion determination of the sum of slow phases/60 sec was more exact than determination of the speed of the slow phases

RESULTS

Two hundred out of these 784 patients showed a nystagmographically clear vestibular spontaneous nystagmus being present either with closed or open eyes in darkness or with both conditions but not with visual fixation and when looking straight ahead

Patients with an ocular pendular nystagmus were eliminated 42% were men 58% women Fig 2 depicts age incidence Surprisingly the group of the 31–35 year old patients was almost as large as the group of the 61–65 year old patients In 45% nystagmus was directed to the left in 55% to the right In 69% the etiology of spontaneous nystagmus was a peripheral vestibular lesion and in 22% a central vestibular one In 9% it was not possible to localize the disturbance definitely

In 200 patients in supine position spontaneous nystagmus was demonstrated

only with open eyes in darkness	47 times
only with closed eyes	60 times
both with closed and open eyes	93 times

As seen in Fig 3*a b* the nystagmus which is present with only open or only closed eyes need not be of low intensity (parameter in Fig

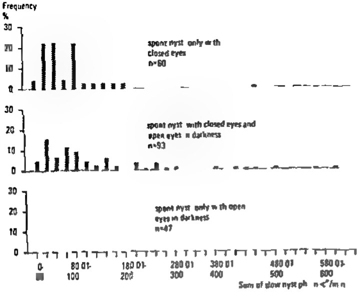

Fig 3a

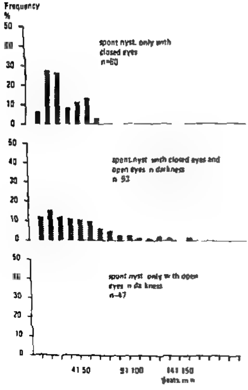

Fig 3b

3*a* sum of slow phases/60 sec, in Fig 3*b* beats/60 sec), however, the ratio of a highly intensive nystagmus is somewhat higher in the group of patients showing nystagmus with open and closed eyes In the case of the 93 patients showing spontaneous nystagmus in both conditions the latter was more intense in 72% with closed eyes and in 28% with open eyes in darkness In these 93 patients nystagmus intensity with open eyes in darkness was 72% (beats) and 68% (sum of slow phases) of the intensity with closed eyes

Figs 4*a* (parameter sum of slow phases 60 sec) and 4*b* (parameter beats/60 sec) demonstrates the intensity distribution of the spontaneous nystagmus in all patients A weak spontaneous nystagmus up to 30 beats/60 sec or up to 60 degrees/60 sec (see also Fig 3*a*) occurs more frequently with open eyes in darkness, whereas a highly intense nystagmus is rather recorded with closed eyes In addition for comparison in Fig 4*a* the physiological spontaneous nystagmus of 120 healthy individuals in the supine position is demonstrated (Mulch & Trincker, 1975) A nystagmus of an intensity exceeding 120 degrees/60 sec was only found in patients and not in healthy persons

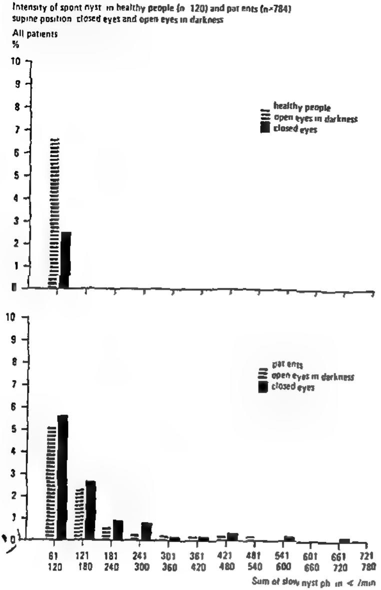

Fig 4a

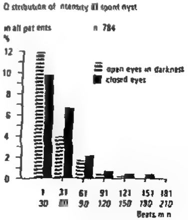

Fig 4b

Fig 5 shows the behaviour of spontaneous nystagmus in cases of peripheral vestibular and central vestibular origin As to peripheral vestibular disturbances ($n=138$) nystagmus is present in about half of the patients with both open and closed eyes Nystagmus with only open or only closed eyes appears approximately in the same range This behaviour of spontaneous nystagmus is nearly the same in various diseases, such as isolated vestibular loss or labyrinthitis In contrast, about half of our patients with central vestibular lesions

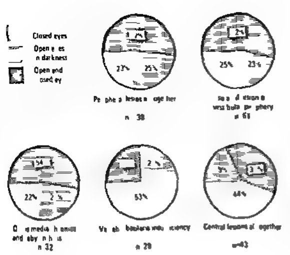

Fig 5

(*n*=43) had nystagmus with closed eyes only The intensity of the spontaneous nystagmus of peripheral and central vestibular genesis showed no significant difference The above mentioned different reaction cannot be explained by this

Fourteen patients underwent follow up examination 1–2 times within a time period of 7–143 days (42 days on average) In two thirds of our cases in the second and third examination the reaction of the spontaneous nystagmus with open and closed eyes was identical with that of the pre-examination In cases where the spontaneous nystagmus in the follow up examination was different from the primary examination the first electronystagmography was dated back longer (*n*=56 days) than in those patients with identical reactions (*n*≈35 days) The results were best reproducible in those cases where the spontaneous nystagmus was present with only open or only closed eyes (*n*=5) In this cases the result of the follow up examination was exactly identical with that of the first electronystagmography in all patients

DISCUSSION

In the clinical observation of the vestibular spontaneous nystagmus we use Frenzel s glasses which impede visual fixation However Frenzel s glasses themselves may inhibit spontaneous nystagmus as electronystagmographic investigations with open eyes in darkness disclosed (Rossberg 1954 Aschan et al 1956 Preber 1957 Frenzel & Tonndorf 1957 Aschan 1960) On the other hand a nystagmus present with open eyes in complete darkness may be activated by eye closure This applies to both the spontaneous nystagmus (Rossberg 1954) and the vestibular nystagmus induced by rotatory (Rossberg 1954) and caloric stimulation (Mahoney et al 1957 Megighian & Waldecker, 1961, Naito et al 1963) Since recording with closed eyes is in any case the simpler of both possible methods to exclude visual fixation it is mostly preferred in electronystagmography

We found some remarks in the literature concerning inhibition of a vestibular spontaneous nystagmus by eye closure just as in our aforementioned observation

True Megighian & Waldecker (1961) observed that all muscular (open eyes in darkness) and retinal (light effect) impulses originating from the ocular part of the optic vestibular reflex arc inhibited the vestibular nystagmus but on the other hand they saw also test persons with a disturbed caloric nystagmus due to eye closure Dix et al (1963) reported that in cases of brain stem lesions due to tumorous pressure in the level of the vestibular nuclei a spontaneous nystagmus existing at visual fixation might disappear after eye closure but reappear with open eyes in darkness It was Decher (1965) who determined the rotatory stimulus threshold in healthy individuals and patients with disturbances of equilibrium In this connection the threshold for nystagmus with open eyes in darkness was lower than that with closed eyes According to Decher with open eyes in darkness there were more regular recordings and a better evaluation of the amplitude and the beats

Kornhuber (1966 1974) pointed out that not only in patients with brain stem lesions but also in fatigued healthy individuals the inten

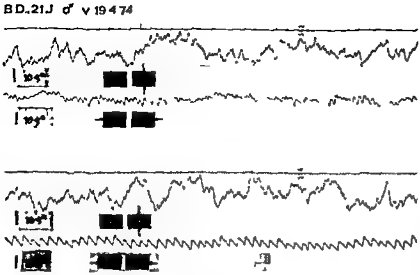

Fig 6 Spontaneous nystagmus showing better regularity and higher amplitude by open eyes in darkness

sity of experimental nystagmus might decrease due to eye closure because the quick phases then lack facilitation by gaze intention In addition nystagmus might be completely eliminated by eyelid pressing

Smith et al (1973) described a patient who obviously had a spontaneous nystagmus with open eyes However it was not present during electronystagmography with closed eyes A caloric reaction was recorded only when pressure was applied on both eyeballs The authors called this an unusual kind of an arousing mechanism

Tjernstrom (1973) communicated the results of his comparative study on the rotatory nystagmus recorded with open eyes in darkness and with closed eyes In a greater number of the test persons the nystagmic reaction was only present with open eyes in darkness With closed eyes the nystagmus was missing as long as the bulbus remained elevated

Our systematic examinations reveal that in a rather high percentage of our patients the vestibular spontaneous nystagmus is inhibited or even completely eliminated by eye closure We observed quite similar findings in the physiological horizontal spontaneous nystagmus (Mulch & Trincker, 1975) It is true

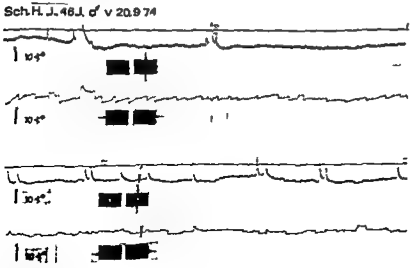

Fig 7 Spontaneous nystagmus recognizable with closed eyes only

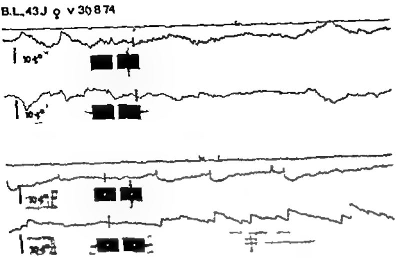

Fig 8 Spontaneous nystagmus with open eyes in darkness only

that the afore described case of spontaneous nystagmus showing a high intensity with open eyes in darkness and being completely suppressed by eye closure, is rather an exceptional one In about 1/4 of our patients, however, spontaneous nystagmus occurred only with open eyes (Figs 3 and 8) and in more than 1/4 of the patients with nystagmus present with both open and closed eyes, the latter was more intensive with open eyes in darkness That a spontaneous nystagmus of low intensity is more frequently recorded with open than with closed eyes (Figs 3 and 4) can be related partly to the fact that the electronystagmographic recording with open eyes is less disturbed by artefacts, etc This means that a nystagmus of slight angle speed can more easily be recognized Kirstein & Schopfer (1956) as well as Decher (1965) have pointed out this phenomenon

The relatively frequent intensity decrease of spontaneous nystagmus due to eye closure might be partly attributed to the vigilance reduction indicated in the EEG by the increase of alpha activity (Mahoney et al 1957) As is generally known this increase in alpha activity includes a decrease of vigilance Other authors consider that nystagmus intensity is a more sensitive parameter of vigilance alteration than the EEG (Collins et al 1961) In addition during the caloric vestibular response with closed eyes there appear nystagmus pauses which can be interrupted by unspecific arousing stimuli (Megighian & Waldecker 1961, Collins et al , 1962, Sokolovski, 1966, Barber & Wright, 1967 Gillingham 1969) Probably a lower vigilance level with closed eyes is still present despite mental alerting On the other hand, Torok (1970) stated that mental activity during caloric vestibular examination does not elevate nystagmus intensity significantly when recording with open eyes in darkness Thus, a higher vigilance level can also be produced by the conscious voluntary action of keeping the eyes open in darkness However, vigilance alterations entail nystagmogenic threshold changes in the neuron synapses of the mesencephalic reticular formation (Trincker & Sieber, 1962, Trincker, 1965) Besides, it seems to be essential that when recording with open eyes in darkness, visual intention and searching eye movements persevere Consequently, Tjernstrom (1973) was able to show with d c recording that the perrotatory nystagmus was especially inhibited when the bulbus remained elevated after eye closure He was thus able to prove that there is a close relationship between eye elevation after lid closure and a degree of nystagmus inhibition

Hall (1936) described how in about 90% of his test individuals the bulbi were elevated at voluntary lid closure, whereas during sleep the bulbi were frequently found in a normal position Fluur & Eriksson (1961), on the other hand, reported—unfortunately without indicating the time constant used—that after initial elevation the bulbi always returned to mid-position Tjernstrom (1973) demonstrated that at d c recording the bulbi remain partly elevated and partly return to their normal position

Consequently, closed and open eyes in darkness represent two quite different "functioning states' of the oculomotor system However, it is not yet clear why in the very same functioning state in one individual the spontaneous nystagmus is inhibited while it is stimulated in another one The site of lesion within the vestibular system seems not to be responsible for it, at least not in the case of peripheral vestibular disturbances, since in 22% of our patients with labyrinthitis, spontaneous nystagmus appeared only with closed eyes and in 25% only with open eyes in darkness (Fig 5)

We are not able to comment upon the find-s of Dix et al (1963) who have described erent reactions of the spontaneous nys-mus dependent on the corresponding eight of brain stem lesions Among our 784 atients only one had a surgically proven brain mor The central vestibular disturbances found in our patients were mainly attributed to lesions due to vertebral basilar insufficiency and encephalitis disseminata Thus an exact localization of the brain stem disturbances was not possible Although concerning the statistical average, the nystagmus of peripheral origin behaved differently from that of the central vestibular origin (Fig 5) we do not deem it useful for topodiagnostic purposes (Conraux et al , 1969)

CONCLUSIONS

1 A nystagmus inhibition by lid closure must be considered if there is no spontaneous nystagmus with electronystagmography, although clinical symptoms of a vestibular disturbance are present This is also true in the case of mental alerting

2 In electronystagmography with both open and closed eyes in darkness, the search for spontaneous and positional nystagmus should be performed

3 The diagnosis of "non-excitability of both labyrinths" is probably established too frequently it can only be ascertained when vestibular excitability responses are absent at electronystagmography with eyes open in darkness, or behind Frenzel's glasses

ACKNOWLEDGEMENT

We wish to thank Prof Dr Kornhuber Department of Neurology The University of Ulm for his valuable suggestions during the course of our study

ZUSAMMENFASSUNG

Innerhalb eines Jahres (September 1973 bis September 1974) wurden insgesamt 784 Patienten mit ENG untersucht *Es handelte sich um Patienten die zur Abklarung von Gleichgewichtsstorungen Schallempfindungsschwerhörigkeit und/oder Tinnitus zu uns kamen* 200 davon wiesen einen eindeutigen vestibularen *Spontannystagmus (SN) in Ruckenlage auf Okularer Pendelnystagmus* wurde eliminiert Anhand dieser 200 ENG s wurde der Einfluß des Lidschlusses auf den pathologischen vestibularen SN analysiert Nimmt man den statistischen *Mittelwert so wurde ein SN der bei offenen Augen in Dunkelheit* bestand durch Lidschluss intensiviert Differenziert man so wird deutlich dass 24% der Patienten uberhaupt nur bei offenen Augen in *Dunkelheit einen SN aufwiesen 30% nur bei geschlossenen Augen* Aus dem Auftreten des SN nur bei offenen Augen im Dunkeln oder nur bei geschlossenen Augen oder bei beiden Ableitungsmodi lassen sich u E jedoch *keine topodiagnostischen Schlusse ziehen* An die Möglichkeit einer Nystagmushemmung durch Lidschluss muss gedacht werden wenn anamnestisch eine vestibulare Storung naheliegt bei geschlossenen Augen im ENG ein SN jedoch fehlt Beispielhaft werden die klinischen Beobachtungen an einem Patienten im Detail geschildert Zur Vermeidung von Irrtumern sollte nach der unerlasslichen Untersuchung mit der Frenzelbrille — falls nystagmographiert wird — sowohl bei geschlossenen als auch bei offenen Augen in Dunkelheit nach einem Spontannystagmus gefahndet werden

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A REEXAMINATION OF "NECK REFLEX" EYE MOVEMENTS IN THE RABBIT

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Abstract Horizontal eye movements of rabbits were recorded during sinusoidal oscillation (15–40° pk pk 0 1–8 Hz) and step displacement of the body in yaw about a fixed head Modulated slow phase eye movements followed all frequencies of stimulus with relatively invariant amplitudes (2–4°) Saccadic movements up to 17° accompanied all frequencies of oscillation and particularly step displacements Saccadic amplitude was unrelated to measurable characteristics of the stimuli but was a function of arousal The latency of any eye movement was a minimum of 80 msec It is concluded that none of the observed eye movements provide stabilization during head movements but are evidence of the contribution of neck information to general mechanisms of whole body orientation

e eye movements produced by stepwise an lar displacements of the neck of the rabbit re first reported by Bárany (1906) In particular upon displacing the animal s body, in yaw, about a fixed head, he observed the eye to make a distinctive rotation in the same clockwise direction as the body displacement This so-called 'static neck-eye reflex' was elaborated by Magnus (1924) and de Kleijn (1918, 1921, 1922) Of particular importance is the study of de Kleijn (1921) which introduced modern recording techniques in the form of precision photography Hughes (1971) using photography and videotape recordings reconfirmed the findings of de Kleijn and extended the work to include considerations of voluntary head movements and how vestibular and neck "reflexes" might be involved in the regulation of corresponding eye movements Suzuki (1972) used frequency response analysis to study the "reflex" There is a distinction between static and dynamic aspects of the "reflex" The large amplitude eye movement produced during stepwise displacement of the body about a fixed head is saccadic in nature and comprises the "static" aspect of the response In addition, small 'slow-phase' movements are produced which, for sinusoidal oscillation, are related to the forcing function (Suzuki, 1972, Takemori & Suzuki, 1971) It was the intention of the present study to extend the range of stimuli beyond those used previously, paying attention to the starting transients as being of particular value in indicating the nature of the reflex and to achieve a high degree of temporal resolution

Recent experiments by Gresty (1974) Gresty & Benson (1975) on man and Gresty (1975) on the guinea pig have shown that a wide range of voluntary reflex and passive displacements of the head are executed with similar frequency components The principal component is about 3 Hz for man and 4 Hz for guinea pig In the light of this information the present experiment set out to measure the typical amplitudes and time courses of head movements produced volitionally by the rabbit and used these measures to define the experimental stimuli The analysis was restricted to movements in yaw produced whilst the animal

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was in its "normally seated" or "crouching position"

EXPERIMENTAL METHODS

Two young, male "Australian white" rabbits, weighing 2 5 and 2 8 kg were selected for their distinctive reflexes With the rabbit in a sitting position, the eyes were photographed to determine the axes about which they rotated during angular displacements of the body with respect to the head and during oscillation of the body and head in yaw Almost pure horizontal movement was seen This result was corroborated in a third rabbit in whom the right eye was tattooed and then photographed

The rabbits were anaesthetized with ketamine hydrochloride and the right eye was implanted with chlorided silver ball electro-oculographic electrodes (E O G) to detect horizontal and vertical movement Steel screws were implanted in the bone of the nasal prominence to anchor a miniature four-way electrical socket and two steel bolts The E O G leads were taken subcutaneously to the socket The bolts enabled the head of the animal to be restrained

Eye movement recording using a photodetector

The photooptical eye movement recording technique employed an infra-red light emitting diode (General Electric, SSL-315) which produced a narrow beam of light with peak spectral emission at 940 manometers The device was implanted on a small cylinder of stiff rubber the other end of which was a suction cup of the type used to pick up contact lenses Two extremely fine wires powered the device from an adjacent battery Under local anaesthesia the suction cup was pressed onto the right eye of the rabbit in the region of the iris, hence it did not completely obscure vision The beam from the photodiode projected normally away from the surface of the eye and at a distance of 2 cm encountered the sensitive surface of a United Detector Technology (Schottky barrier) SC/10 or SC/25 photodetector which was mounted on a micromanipulator The axes of the detector were oriented horizontally and vertically The output from the photodetector is proportional to the position in the x and y axes of the centroid of any light incident upon the detector surface In the present application the output of the detector during eye movement is proportional to the tangent of the angle through which the eye moves Calibration of this technique was made by moving the detector through a known distance whilst the eye was stationary, observing the output and using the tangent relationship to calculate an equivalent eye displacement In addition, scale photographs were taken of the eye using the suction cup as a marker These procedures also served to calibrate the E O G

Voluntary head and eye movement recording

The rabbit was placed in a narrow box which permitted little movement The head projected freely out of one end of the box A small light bulb was attached to the bolts implanted in the skull A Schottky barrier photodetector was placed in the focal plane of a 35 mm camera, positioned to view the head directly from above The output from one axis of the detector was sinusoidally related to angular displacements of the rabbit's head The recordings were calibrated using either scaled and synchronized photographs of the top of the rabbit's head, or by manual displacements of the rabbit's head

Vestibular stimulation

The animal's body was tightly bound, mummy fashion in a strong linen cloth and strapped onto a small turntable The head of the rabbit was fixed in a rigid framework and held so that the axis of rotation passed through the skull which lay in a "normally seated" attitude A position potentiometer attached to the turntable indicated angular displacement of the rabbit in yaw

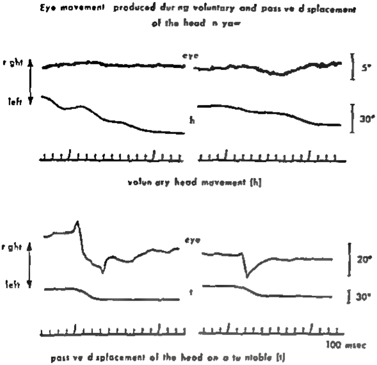

Fig 1 Voluntary head and eye displacements in yaw were recorded with the torso of the rabbit placed in a narrow box having the protruding head free to move Passive displacement of the head in yaw was provided by tightly wrapping the torso of the rabbit and fixing his head to a rigid framework on a turntable The eye movements were recorded using the E O G technique

Sources of error

It was estimated that the techniques described for calibrating head and eye movement were accurate to between 5 and 10% Importantly the photodetector technique gives *consistent* records of eye displacement in addition to very high temporal resolution It seemed likely that vibrations set up in the body of the animal could be a source of interference This was tested by pummelling the animal s body The resulting artifacts were surprisingly small and looked nothing like the experimental recordings

Apparatus for oscillation of the body in yaw

This consisted of a rectangular aluminium plate free to rotate in the horizontal plane about a potentiometer shaft fixed to the middle of a short side The opposite side was yoked via a variable eccentricity cam to a fractional horsepower d c motor

Experimental procedure

Initially each rabbit was subjected to the experimental situation designed to record voluntary eye and head movements Typically when placed in the box the rabbit would be intimidated for a few minutes then would begin to move his head in an exploratory manner and eventually try to walk out of the box Each animal was inspected several times in this situation until general patterns of movement were established It was found that voluntary angular displacements of the rabbits head were sigmoid in shape and executed in about 200–400 msec with amplitudes ranging between 15° and 30° (Fig 1) Transient angular displacements of similar amplitudes and time courses were used as vestibular stimuli when the rabbit was passively rotated on the turntable

In order to provide passive rotation of the torso in yaw about the neck and head the body of the rabbit was bound and strapped to the aluminium plate The head was bolted in the normally seated attitude to a rigid framework with the atlanto axial atlanto-occipital region of the neck directly above the axis of rotation of the plate Eye movement recording devices were then positioned

Two forms of stimuli were used The body

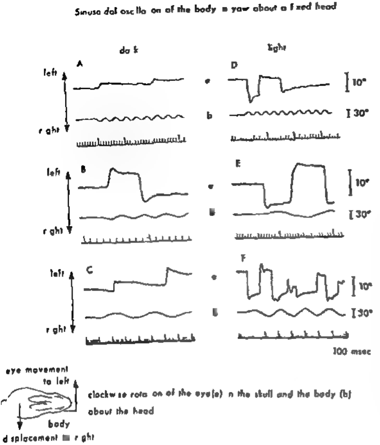

Fig. 2. The head of the rabbit was fixed to a rigid stationary frame work. The body was tightly wrapped and strapped to a horizontal plate which oscillated in yaw about a perpendicular axis passing through the animal's neck. The infra red photodetector technique was used to record eye movement. Time markings are at intervals of 1 sec and 100 msec.

of the animal was sinusoidally oscillated in yaw at frequencies varying systematically from 0.1 to 8 Hz at peak to peak amplitudes of 15°, 20°, 30° and 40°. The animals were also subjected systematically to stepwise displacements of the body in yaw at amplitudes up to 40° and time courses similar to those of voluntary head movements. Each rabbit underwent this regime on two separate occasions. On each occasion he was subjected in both total darkness and under normal laboratory illumination to the complete range of frequencies and amplitudes of stimuli. High frequency stimuli were applied for periods of about 5 seconds or until the rabbit displayed some distress. Low frequency stimuli were employed for suitably longer times. Concurrent E.O.G. and photodetector eye movement recordings were taken. Signals from eye and head movement recording devices were processed using differential d.c. amplifiers with a passband up to 100 Hz. The outputs of the amplifiers were stored on magnetic tape.

RESULTS AND COMPARISON WITH PREVIOUS WORK

Voluntary head and eye movements

Representative samples of voluntary head and eye movements in yaw are illustrated in Fig. 1. The rabbits exhibited a preference for executing wide angle head displacements in yaw as a series of smaller steps. The steps were sigmoid in shape and endured for between 200 and 400 msec. The principal frequency with which the head movement was executed lay between 2.5 and 5 Hz.

Very little eye movement occurred during the head movements described above (Fig. 1). When the animals became active to the extent

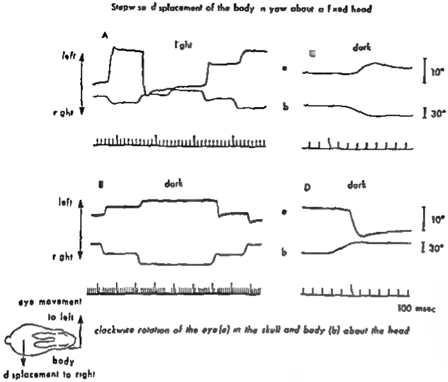

Fig 3 The experimental technique is the same as that employed in Fig 2 The plate was driven using step displacements with similar amplitude and velocity characteristics to those of voluntary head movements

that it was impossible to monitor their movements adequately then eye movement did occur These closely resembled vestibular reflex eye movements and were unlike the eye movements which accompany voluntary head rotation described in man (Gresty, 1974), in primates (Bizzi et al , 1972) and in guinea pig (Gresty, 1975) This finding is at variance with the data of Hughes (1971) but is in agreement with the views of Brecher (1936) The differences of opinion may be the result of selecting representative head movements or of the resolving powers of the respective recording techniques employed

Eye movements evoked by passive rotation of the head in yaw

Horizontal eye movements consequent upon transient passive rotations of the rabbits in yaw are illustrated in the lower traces of Fig 1 In both examples the eye movement commences with a slow phase compensatory displacement which is synchronized with the onset of the head rotation and which rotates the eye in the opposite direction to that of the head The slow phase gives way to a reflex saccade or "fast phase" of vestibular nystagmus which may have a latency measured from the beginning of the head movement as short as 50 msec Following the saccade there is a further period of slow phase movement which may last long after the termination of the head movement The irregular recording of figure 1 was taken when the animal was greatly excited Despite this drawback the basic profile of the response is clearly present Such a profile is very similar to that of the responses seen in man during transient passive rotation in yaw (Gresty, 1974)

Sinusoidal oscillation of the body in yaw about a fixed head

Sinusoidal slow phase eye movements with amplitudes varying from about 2° at the high frequencies to 3–4° at low frequencies (Fig 2C) were present most of the time at all frequencies of oscillation both in darkness and

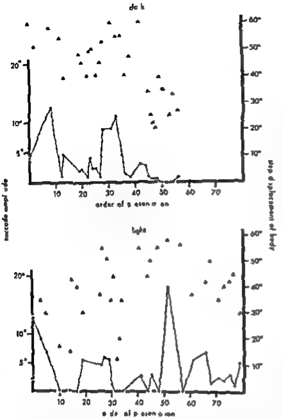

Fig 4 The amplitudes of consecutive stepwise displacements of the body of a rabbit in yaw about a fixed head and amplitudes of contingent eye movements (see Fig 3) under the conditions of darkness and laboratory illumination. Circles and triangles represent eye and body displacements respectively. The horizontal axis is scaled in seconds

under laboratory illumination. They varied very little in any measurable characteristics. Fig 2E shows that on occasions the response could be absent. *The direction of such steady state slow phase movement was always in the opposite clockwise sense to that of the body rotation.* The precise phase relationship between the slow phase movement and the body displacement remains obscure because no direct measurement can be made of the timing of the mechanical events which activate the receptors whose activity is responsible for the reflex. In the light of the experiments of McCough et al (1951) the principal receptors involved are probably those of the atlanto-axial and atlanto occipital joints

Saccades were produced at irregular intervals during the oscillation. In amplitude they varied between 4° (Fig 2A) and 17° (Fig 2E). As a general rule the saccade was executed in *the same clockwise direction as that of body* movement (e.g. Fig 2B, D, E, F) and hence resembled the "static neck-eye reflex". Occasionally, as in Fig 2C, the saccade could be executed in the opposite clockwise sense. At low frequencies of oscillation the saccades could be evoked during almost every cycle of oscillation (Fig 2F), in contrast, high frequency oscillation evoked few saccades and often only at the onset of the stimulus (Fig 2D). The saccades appeared more readily in the light than in darkness (contrast Fig 2A, B, and C with D, E, and F) and at high frequencies of oscillation in darkness they were relatively small in amplitude (Fig 2A). At times *the animals exhibited a distinct preference for* executing saccades in one direction only (Fig 2A, C)

Following the termination of a saccade it

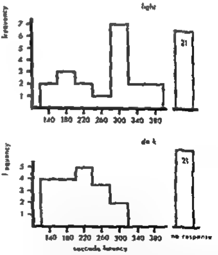

Fig 5 Frequency histograms of the latencies of saccadic eye displacements contingent upon 40 successive step-*wise displacements of the body of a* rabbit in yaw about a fixed head for both light and dark conditions. The data were selected to equate numbers of "no responses" in an attempt to control for the effects of arousal. Ab scaled in milliseconds

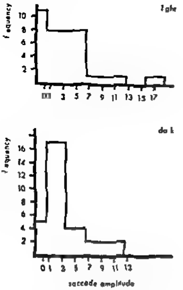

Fig 6 Frequency histograms of the amplitudes of eye movements plotted in Fig 4 Abscissa scaled in degrees

was common to observe long, slow phase, drifting movements of the eye, presumably returning it to some medial position (Fig 2C, D) A careful examination of the starting transients of the responses revealed that they frequently commenced with a saccade (Fig 2A, B, D, E, F) which had an average latency of 200 msec When the response commenced with slow phase movement (Fig 2C) the *shortest* ncy observed was 80 msec These aspects the response will be examined closely in the next section

Stepwise displacement of the body in yaw about a fixed head

Saccadic eye movements were produced by stepwise body displacements more consistantly than by sinusoidal oscillation Consequent upon a body displacement the eye made either a slow phase movement which approached the velocity of saccades (Fig 3C) or executed an identifiable saccade (Fig 3D) which could be preceded by some form of slow phase movement (note the last saccade made in Fig 3A) The distinction between the high velocity slow phase movements and saccades is unclear On occasions the eye would do nothing at all during a body displacement (Fig 3A) Slow phase movements observed were always in the *same clockwise direction* as the body displacement, quite unlike the steady state slow phase movements seen during sinusoidal oscillation

Saccadic amplitude appeared to be a variable independent of the duration of the body displacement Figure 4 shows the amplitudes of step displacements and corresponding responses plotted as a function of their temporal order of occurrence The correlation coefficient between amplitude of step displacement and eye movement is 3.5×10^{-3} for the dark condition and 7×10^{-5} for the light condition The values of the coefficients indicate that the amplitude of the response is totally unrelated to the stimulus amplitude

It is apparent that movements of a given approximate amplitude occur in runs and are unrelated to the amplitude of the stimulus This is evidence that the saccadic amplitude is related to the state of arousal of the animal

The latencies of eye movements produced in response to stepwise body displacement were carefully examined Frequency histograms of the data are presented in Fig 5 The latencies were measured to the first detectable response whether saccadic or slow phase The mean latency for the light condition was 230 msec with a range of 80–360 msec The mean latency for the dark condition was 207 msec with a range of 80–320 msec These measurements suggest a shortest latency response under any conditions of the order of 80 msec

The variety of amplitudes of eye movements produced under both conditions are presented in the frequency histograms of Fig 6 Although large distinctive eye movements (up to 17°) could be evoked, it was found that on repeated testing rather small amplitude movements predominated

A general characteristic of saccadic responses was that they were strongly affected by the state of arousal of the rabbit At times noise stimuli were applied to "wake the animal up" and facilitate the response For these reasons a judicious selection of the data was

employed in the comparative analysis. This does not affect the important measures such as range of latency.

DISCUSSION

Several features of the saccadic eye movements produced by body displacement indicate that the nature of the mechanism responsible is highly complex and is geared more to the general orientation of the animal rather than to specific problems of stabilizing the eye in the head. These features are the following. The saccades are unrelated to amplitude or velocity of body displacement. They wax and wane in amplitude, contingent only upon the fact that a disturbance has occurred. Such characteristics imply that the saccades are a function of the state of arousal of the animal. An animal can show a high degree of directional preference in executing saccades and the direction of execution is occasionally opposite to that usually exhibited for a given direction of body movement (quite unlike saccades of vestibular origin). This is evidence that the reflex is susceptible to modification by general states of the animal or perhaps by the environmental context.

The latencies (200 msec) at which saccades were evoked by step stimuli are much longer than those of the vestibular ocular reflex. They may commence when the motion stimuli are almost over. It would seem therefore that they could have no dynamic role to play in stabilization during head movement.

It was found that the saccades were somewhat smaller and less frequently evoked in the dark than in the light, both being characteristics which suggest that the saccadic eye movements were facilitated by the presence of vision on a visual environment. This may be taken as further evidence that the saccades have an orienting function rather than a stabilizing one.

It is difficult to see how neck reflex saccades could work systematically to stabilize the visual world during the course of the various body displacements studied. A more reasonable function which may be ascribed to them is that of reorientation following disturbances. The saccades display characteristics of movements which are the product of arousal and reorientation. This explanation of their origin and function is congruent with all the features detailed above.

The present experiment did not produce such informative evidence about the nature and function of slow phase eye movements. With regard to the mechanism of their production, electrophysiological data is incongruent with the behavioral observations. Hikosaka & Maeda (1973) by electrically stimulating C2 and C3 evoked EPSPs and IPSPs in the abducens motoneurones of the cat with latencies as short as 2.8 msec. The authors conclude that "the cervico-ocular pathway ... may function conjointly with the vestibulo-ocular reflex to carry the compensatory eye movement". However we observed no eye movement produced by the body displacement with a latency less than about 80 msec, 30 times longer than the electrophysiological latency to an electrical stimulus.

Voluntary head displacements of the rabbit last about 300 msec. It is likely (because of the body's filter characteristics) that passive head displacements are executed in a corresponding frequency range (Gresty & Benson 1975). The vestibular ocular reflex, having an extremely short latency, is capable of operating almost throughout the entire movement. The slow phase eye movements produced by the neck reflex only begin to have behavioral consequences one third of the way through the movement and then are small and unrelated in amplitude to the motion stimulus. *Therefore* it seems unlikely that, under the circumstances delineated above, they may contribute to major eye movements. They may play a part in the control of small subtle compensations used in the "freeze" position (Brecher 1936).

During voluntary movement of the head the rabbit has the ability to suppress vesti

bular-reflex or "neck reflex" eye movements (Fig 1) despite the fact that they appear so strongly during passive manipulation of the animal It seems that during the voluntary movements studied, cervical and vestibular effects are both completely blocked However, during the everyday behavior of the rabbit conditions may be different

The experiments of Cohen (1961) together with the clinical data relating to "cervical vertigo" (Weeks & Travell, 1955, Gray, 1956, Cope & Ryan, 1959) demonstrate the crucially important role that information from the neck plays in whole body coordination and spatial orientation It is a strong possibility that the eye movements evoked by neck reflexes are related more to holistic mechanisms of body geometry and orientation than to the specific tasks of stabilization of the eyes in the head or visual search Working on this hypothesis a suggestion may be offered that the function of the neck reflex saccade is to maintain the maximum area of *useful* visual field

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ZUSAMMENFASSUNG

Horizontale Augenbewegungen des Kaninchens wurden während Sinusreizung (15–40° pk pk 0 1–8 Hz) und Stepreizung um die vertikale Achse bei fixiertem Kopf registriert Die langsamen Augenbewegungen folgten allen Reizfrequenzen unabhängig von der Amplitude (2–4°) Sakkaden (bis 17°) begleiteten die sinusförmige und besonders die stufenförmige Reizung Die Sakkaden amplitude war unabhängig von den messbaren Reizparametern veränderte sich aber mit dem Wachheitszustand Die Latenzzeit aller Augenbewegungen betrug minimal 80 msek Es wird festgestellt dass keine der beobachteten Augenbewegungen eine Stabilisierung bei Kopfbewegungen gewährleistet sondern möglicherweise einen Beitrag der Halsafferenzen zum Mechanismus der Korperorientierung darstellt

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THE ORIGIN OF SLOW POTENTIALS IN SEMICIRCULAR CANALS OF THE FROG

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Abstract Slow potentials generated in the sensory organ of the ampulla were recorded in isolated semicircular canals of the frog by means of fluid electrodes These potentials which may be picked up from the intra ampullar fluid and those from the ampullar nerve appear to be generated at different stages of the process taking place in crista ampullaris Slow intra ampullar potentials apparently reflect receptor potentials of hair cells They are preserved after degeneration of the nerve fibre endings and are relatively insensitive to DNP poisoning their amplitude is maximum at high K^+ concentrations Slow nerve potentials appear to be due to electronic spreading of post synaptic excitatory potentials generated at cytoneural junctions They disappear after degeneration of the nerve fibres in low Ca^{++} and high Mg^{++} solutions and are extremely sensitive to DNP poisoning An analysis of the time-course of the slow ampullar and nerve potentials referred to the discharge of impulses in afferent fibres was performed with a view to interpreting the transduction mechanism of semicircular canals

Studies on semicircular canals have shown that deflection of the cupola is associated with the appearance in the ampullary (Trincker, 1957) and neighbouring structures (Trincker, 1957, Sala, 1965) of slow electrical potentials which also spread to the ampullar nerves (Rapuzzi & Bruschi, 1967) These potentials, sometimes called "ampullar microphonics" are believed to be related to the transduction processes of ampullar receptors, but their precise significance is still unclear

At least two processes in labyrinthine receptors might give rise to slow potentials, i e , membrane potential changes produced by hair bending in sensory cells (receptor potentials) and post synaptic depolarization of the nerve fibre endings, due to transmitter release at cytoneural junctions (generator potentials) Unfortunately no data are yet available on intracellular recordings in ampullar or cochlear hair cells, although Harris et al (1970), by impaling sensory cells in the lateral line organ of the mudpuppy, and Weiss et al (1974), by impaling sensory cells in the basilar papilla of the alligator lizard, were able to record membrane potential changes evoked by hair deflections The potentials recorded by these authors were however very limited in amplitude (less than 3 mV) even in response to stimuli of maximum intensity, so that individual hair cells appeared to be very weak electrical generators In any case, it is possible that the synchronized membrane depolarization of a very large number of hair cells as occurs during cupula deflection, might account for the slow potentials which may be picked up from different structures of semicircular canals

As to the presence of synaptic potentials in labyrinthine receptors, Furukawa et al (1972) and Flock et al (1973) were able to successfully impale the nerve endings of the afferent sensory neurons within the sensory epithelium in the goldfish sacculus and in the lateral line

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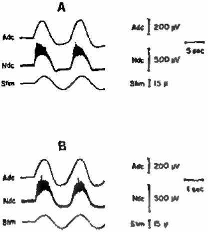

Fig 3 Slow nerve (*Ndc*) and ampullar (*Adc*) potentials in response to sinusoidal stimuli having frequencies of 0 1 Hz (*A*) and 0 5 Hz (*B*) Plunger displacements (*stim*)

changes in amplitude and time course of the evoked d c potentials could be detected as a function of survival time indicating that the responsiveness of the preparation was well preserved throughout the experiment

As Fig 2 shows, ampullar d c potentials were found to have a similar time course but an opposite polarity to the nerve potentials Indeed, during excitatory cupula deflections the nerve electrode revealed a positive potential shift whereas the ampullar electrode be-e negative Changes of opposite polarity observed during inhibitory cupula deflec ns

The polarity of ampullar potentials was unchanged in experiments in which the lead-off electrode was facing the canalicular rather than the utricular side of crista ampullaris This enabled us to rule out any possibility that the potential recorded could be the result of current field modifications in the intra ampullar volume produced by cupula movements In fact if a steady d c source had been present in the cupula its displacements during stimulation would have produced potential variations of opposite signs at electrodes facing the opposite sides of the cupula Therefore in agreement with Trincker's observations it was concluded that intra ampullar recordings reflect actual potential changes in the generator located in the sensory organ of the ampulla in response to excitatory or inhibitory deflections of the cupula The polarity of the recordings indicates that the potential sources are so arranged in crista ampullaris as to make the ampullar nerve positive and the interior of the ampulla negative during excitatory cupula deplections and vice versa during inhibitory deflections

Experiments in which the potentials were picked up from several points of the intra-ampullar volume by means of an exploring glass microelectrode much thinner (∅ 3–5 μm) than those otherwise employed in this study revealed that the amplitude of the recorded potentials was maximum when the electrode was approaching the crista ampullaris This therefore appears to be the site where slow ampullar potentials are generated

A more detailed analysis of the slow potentials may be performed based on the tracing in Fig 3 which refers to the responses evoked by sinusoidal stimuli having the same amplitude but two different frequencies (0 1 and 0 5 Hz) In order to facilitate a comparison between the two recordings in this and the following figures, ampullar tracings are reproduced with inverted polarities vis à vis nerve tracings

It can be seen that at both frequencies the nerve recordings reveal a remarkable dissymmetry making reference to the base line, since the amplitude of the electrode positive deflection corresponding to the excitatory half-period of the sinusoidal cycle is greater than the electrode negative shift, related to the inhibitory half-period This dissymmetry is less evident in ampullary recordings which were found to reflect the sinusoidal trend of the stimulus more closely

A considerable difference can also be evidenced when observing the phase relationship of ampullar and nerve potentials Ampullar recordings appear in fact to be nearly in phase with the stimulus and therefore with cupula deflections whereas nerve potential recordings invariably appear to be phase

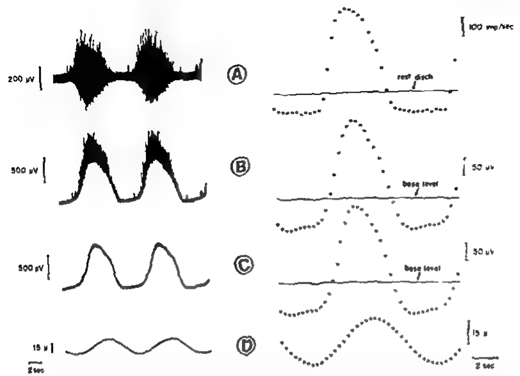

Fig 4 Time-course of slow nerve potentials and mass discharge of impulses evoked by sinusoidal stimulation (*Left*) examples of recordings (*right*) averaging over ten subsequent cycles *A* Mass discharge of impulses (recorded in AC) *B–C* Slow nerve potentials before (*B*) and after spike suppression by treatment with TTX 0 1 µg/ml (*C*) *D* Stimulus (plunger displacements) Base levels were evaluated before stimulation

advanced in comparison with ampullar recordings and the stimulus Both dissymmetry and phase-shift are more evident at low stimulation frequencies and become progressively reduced as the frequency is increased The slow potentials led off from the nerve therefore reflect cupula displacements in a highly distorted way their dissymmetry indicating some degree of rectification and their phase shift a differentiation of the sinusoidal stimulus

The discharge of impulses in sensory fibres superimposed on the slow potentials, may be easily recognized in the nerve tracing in Figs 2 and 3 and appears to be closely related to nerve d c shifts A low frequency discharge of impulses apparently due to spontaneous activity of the receptors, was always observed at rest During the excitatory half periods the discharge increases attained a peak value which coincided with the nerve positive peak of the slow potentials During the inhibitory half period, the discharge is considerably reduced below its resting level, revealing inhibition of the receptors which parallel the nerve negative phase of the slow potential The fairly good coincidence of the positive slow potential peak with the peak frequency of the mass discharge of the impulses is better evidenced in Fig 4 which refers to experiments in which averaging of the impulse discharge and of nerve slow potentials were performed over ten subsequent cycles of sinusoidal stimulation In order to ensure that the presence of the spikes could not have shifted the averaged peak of the nerve potentials a comparison was done with tracings obtained after the spikes had been suppressed by treatment with Tetrodotoxin 0 1 µg/ml (Fig 4C) Since impulse discharge in the ampullar nerve closely parallels slow nerve potential it is accordingly phase advanced in comparison with stimulus and therefore with cupula movements

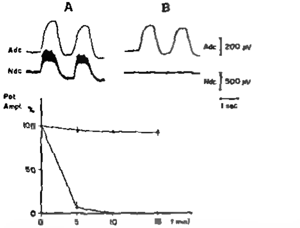

Fig 5 Slow nerve (*Ndc*) and ampullar (*Adc*) potentials in low Ca^{++} (0 1 mM) and high Mg^{++} (10 mM) solutions *Recordings* in plain Tyrode (*A*) 10 min after immersion in modified Tyrode (*B*) *Diagram* peak responses of ampullar (●) and nerve (■) potentials mean values from 5 experiments while preparations were kept in modified Tyrode Values expressed as percentages of controls

Effects of low Ca^{++} and high Mg^{++} solutions

Fig 5 shows an example of the tracings obtained in the experiments intended to influence synaptic transmission in ampullar sensory organ by changing Ca^{++} and Mg^{++} concentrations in the Tyrode bath

Ca^{++} was reduced to 0 1 mM and Mg^{++} increased to 10 mM i e at levels which are known to block transmission completely in many chemical synapses (Katz & Miledi, 1967)

It can be seen that, after immersion in modified Tyrode, the slow nerve potentials are depressed along with the propagated spikes at an early stage until both disappear completely in about 10 minutes Conversely ampullary potentials are fully preserved or only slightly reduced over the same period The changes in amplitude of the ampullar and slow nerve potentials produced by modified Tyrode as a function of time are better illustrated in the graphs in Fig 5 which refer to the mean peak amplitudes of responses obtained from the entire series of these experiments The effects of changing Ca^{++} and Mg^{++} concentrations are easily reversible since potentials can be promptly restored to their original amplitude by washing the preparations with plain Tyrode

Effects of K^+

The tracings reported in the upper part of Fig 6 refer to an experiment in which K^+ concentration in the bath was progressively increased from 0 to 13 mM

It will be seen that even in the absence of K^+ (Fig 6A) stimulation evokes both slow nerve and ampullar potentials having a similar time course but a lower amplitude than those observed in unchanged Tyrode (Fig 6B) Striking changes in the potentials were however produced by increasing K^+ concentration from 2 6 to 13 mM (Fig 6C) K^+ rich solutions proved in fact able to suppress the

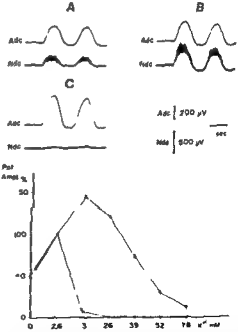

Fig 6 Amplitude of slow nerve (*Ndc*) and ampullar (*Adc*) potentials as a function of K^+ concentration in the bath *Recordings* A $[K^+]$=0 mM B $[K^+]$=2 6 mM C−$[K^+]$−13 mM *Diagram* peak amplitude mean values from 5 experiments of ampullar (●) and nerve (■) potentials versus K^+ concentration Values are expressed as percentages of those observed in normal Tyrode ($[K^+]$= 2 6 mM) Slow potentials were measured 15 min after the application of the solution

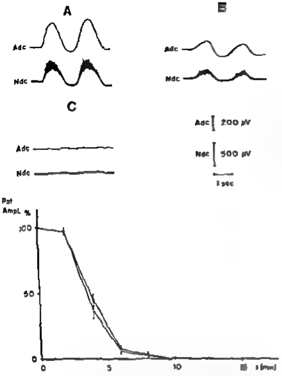

Fig 7 Effects of *d* Tubocurarine (200 µg/ml) on slow nerve (*Ndc*) and ampullar (*Adc*) potentials *Recordings* control (*A*) 3 min (*B*) 5 min (*C*) after exposure to curare *Diagram* peak amplitudes mean values from 5 experiments of ampullar (■) and nerve (O) potentials as percentages of controls

nerve potentials almost entirely, whereas ampullar potentials were not only preserved but even enhanced, in the presence of an excess of K^+

The graph in Fig 6 refers to the mean values obtained from the whole series of these experiments It plots the peak amplitudes of the slow potentials in response to sinusoidal stimuli versus K^+ concentration in the bathing fluid It can clearly be seen that when K^+ concentration is increased the nerve potentials sharply decrease, whereas the ampullar potentials increase to attain a maximum amplitude at K^+ concentration of about 15 mM. Higher K^+ solutions, however, proved able to depress even the ampullar potentials which were virtually suppressed at the highest K^+ concentrations tested (78 mM)

The effects of the highest K^+ concentrations on the slow potentials were almost impossible to reverse by washing with plain Tyrode

Effects of D-Tubocurarine and 2 4 Dinitrophenol (DNP)

Fig 7 shows an example of the tracings obtained in one of the experiments in which D-Tubocurarine was added to the Tyrode bath at a concentration of 200 µg/ml

This drug produced a prompt depression in both ampullar and nerve potentials which appeared 1–2 min after administration and led to total suppression of the slow potentials in about 5 min

The decay in peak amplitude of the slow potentials as a function of the exposure time to D-Tubocurarine, is reported in the graphs in Fig 7 which refer to the mean values of 5 similar experiments

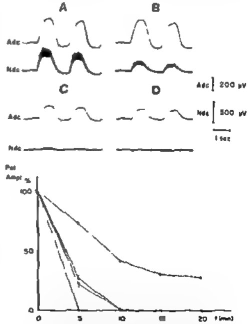

Fig 8 Effects of DNP on slow nerve (*Ndc*) and ampullar (*Adc*) potentials *Recordings* control (*A*) 3 min (*B*) 5 min (*C*) 10 min (*D*) after exposure to DNP (0 3 mg/ml) *Diagram* peak amplitudes of slow potentials as a function of time in the presence of DNP at different concentrations Values are expressed as percentages of controls Adc DNP 0 3 mg/ml (□) Ndc DNP 0 3 mg/ml (■) Adc-DNP 1 mg/ml (▲) Ndc DNP 1 mg/ml (●)

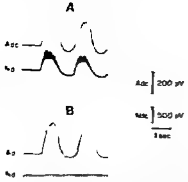

Fig 9 Slow potentials after denervation of crista ampullaris *A* control *B* 10 days after sectioning of the ampullar nerve

Complete recovery from the effects of D-Tubocurarine was easily obtained after about 15 min of washing

The tracings in Fig 8 refer to the effects of DNP at a concentration of 0 3 mg/ml It will be seen that the drug produced a progressive depression of the nerve potentials which was already evident only a few minutes after its addition to the bath The depression resulted in complete disappearance of the nerve potentials within 10 min

The blocking action of DNP was less evident on ampullar potentials which were only ially reduced by the drug at the dose of ml Complete suppression of these potens could only be achieved by raising the NP concentration to 1 mg/ml

The development of DNP blockade as a function of time is reported in the graphs in Fig 8 in which the mean values potential peak amplitudes from groups of five experiments are reported

The effects of DNP are almost irreversible at the doses employed it being impossible to recover the slow potentials even after repeated washings

Effects of denervation of the crista ampullaris

The tracings in Fig 9 were obtained from a semicircular canal preparation ten days after section of the ampullar nerve Histological examinations evidenced complete degeneration of the nerve fibres and of their endings in contact with hair cells The structure of the sensory epithelium was however well preserved

It may be seen from the tracings that neither slow potentials nor propagated spikes can be detected in the nerve recordings By contrast the ampullar potentials are fully preserved and their amplitude is similar to that observed in control experiments

DISCUSSION

The results of the present experiments provide further evidence that the slow potentials which may be picked up both from the intra-ampullar fluid and from the ampullar nerve closely reflect the processes which take place in receptors of crista ampullaris during cupula deflections However slow potentials led off from the nerve and those picked up from the intra ampullar fluid were found to differ in several respects suggesting that they are generated at different links in the neural chain underlying transduction processes

The slow nerve potentials can be abolished by low Ca⁺ and high Mg⁺ media and by DNP poisoning namely by conditions which primarily impair transmission at chemical synapses (Beani et al 1966 Katz & Miledi 1967) furthermore the amplitude and time course are not modified after spike suppression produced by Tetrodotoxin Slow nerve potentials can therefore be interpreted as being due to electronic spreading along the nerve fibres of excitatory post synaptic potentials which are generated at the cytoneural junctions after release of chemical transmitter from the outer pole of hair cells In other words slow nerve potentials reflect nerve ending generator potentials and this explains the closer relationship of these potentials to the discharge of impulses in the afferent nerve fibres

Slow potentials recorded from the intra ampullar fluid were found to be more closely

related to cupula deflections (Schmid et al 1973) and several findings suggest that they are generated in the hair cells of the sensory epithelium thus reflecting true receptor potentials Ampullar potentials are in fact well preserved after the nerve fibre endings have completely degenerated and are unaffected by low Ca^{++} and high Mg^{++} solution Furthermore they increase in amplitude when K^+ concentration in the bathing fluid is raised to levels more than sufficient to abolish nerve potentials Ampullar potentials are therefore supported by a generator whose efficiency is maximum at high K^+ concentrations as might be expected if they originate in hair cells which are normally bathed by a K^+ rich solution namely endolymphatic fluid

However a high K^+ concentration appears to be needed only to provide maximum mechanical responsiveness in labyrinthine sensory cells (Katsuki & Hashimoto 1969) since the experiments have shown that a reduction in K ions can reduce but not suppress ampullar receptor responses Isolated open semicircular canals in fact are known to respond without decay to excitatory stimuli even after several hours of immersion in ordinary Tyrode i e in a low K^+ medium (Taglietti et al 1973) This observation may be related to the data of Matsuura et al (1971) on the sacculus microphonic potentials

As to the nature of the biological battery supporting ampullar slow potentials the relative insensitivity of these potentials to poisoning with DNP suggests that their bioelectric source is not strictly dependent on oxidative metabolism and possibly not even directly related to biochemical processes This is in agreement with the observations of Nishimura & Taglietti (1975) which indicate that there may be a step having a Q_{10} lower than 2 in the processes of ampullar receptors

It is evident that the chain of events underlying hair cell activation is rather complex since in contrast to their relative insensitivity to ion changes and DNP (Matsuura et al 1971) ampullar potentials proved to be sensitive to the depressant action of curare The action of this drug on receptor discharge has been widely studied in semicircular canals by Valli et al (1974) who were able to provide convincing proof that curare blockade acts mainly on hair cells The present results agree with this view in that they indicate that curare is able to suppress rapidly the slow potentials generated by hair cells

Finally it must be stressed that irrespective of experimental conditions the nerve potentials invariably disappear when ampullar potentials are cancelled while the latter can be preserved in most conditions after suppressing the nerve potentials This clearly indicates that slow ampullar potentials are generated uphill vis à vis nerve potentials in the receptor activation sequence

It is open to speculation whether ampullar potentials reflect true potential changes in a source located in the sensory cells or whether they are the result of modulation of the current from a steady generator due to conductance changes in the hair cells as claimed by Davis (1965 1968) The present experiments have not yielded enough information to identify which of these two mechanisms is involved The observation that ampullar potentials are well preserved in open canal preparations obviously confirms that the steady endolymphatic potential is relatively unimportant in the genesis of slow potentials evoked by stimulation However, according to Davis s most recent theory the d c source is likely to be located at the outer pole of the sensory cells and the conductance changes at their hair bearing pole It follows that only experiments in which the fluid inside the canal can be changed independently of the external medium will enable the two processes to be influenced separately

If it is accepted that slow ampullar potentials reflect receptor potentials and slow nerve potentials post synaptic potentials some considerations concerning signal processing in the ampullar sense organ appear to be of interest Although cupula deflections could not

be directly evaluated in the present experiments, the fairly close parallels between ampullar potentials and stimulating fluid displacements suggest that hair deflections in sensory cells are converted into virtually proportionate receptor potentials over a wide frequency range. A considerable distortion intervenes, however, when receptor potentials are converted into generator potentials at synaptic junction where transmission involves partial rectification and a phase-advance of the input signal. This distortion is reflected in the impulse discharge of the afferent nerve fibres. The modalities of transmission at cytoneural junctions and of activation of nerve fibre endings are therefore likely to substantially complement the mechanical properties of the cupula-endolymph system in achieving signal conversion. In particular, these modalities appear to play an important role in accounting for the non linear cupula deflection–impulse discharge transfer function evidenced by Fernandez & Goldberg (1971) and for the well established phase advance of neural signals reaching vestibular nuclei (Milsum & Jones 1969).

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ZUSAMMENFASSUNG

Die langsamen Potentiale, die im sensorischen Organ der Bogengänge des Frosches auftreten, wurden durch flüssige Elektroden abgeleitet. Diese Potentiale, die entweder von der intraampullären Flüssigkeit oder von ampullären Nerven abgeleitet werden können, scheinen von verschiedenen Stufen des Transduktionsprozesses, der in der Crista ampullaris stattfindet, auszugehen. Die intraampullären langsamen Potentiale sind aller Wahr[illegible] gegen DNP-Vergiftung wenig empfindlich, ihre Amplitude ist überdies am grössten bei starken K-Konzentrationen. Die vom ampullären Nerven abgeleiteten langsamen Potentiale scheinen dagegen durch die elektrotonische Propagation der postsynaptisch erregenden Potentiale bewirkt zu werden, die von den zytoneuralischen Verbindungen ausgehen. Sie verschwinden nach Entartung der Nervenfasern in Lösungen mit wenig Ca und viel Mg und sind gegen DNP-Vergiftung sehr empfindlich. Die Analyse des zeitlichen Verlaufs der langsamen Potentiale und der Impulsentladung in den afferenten Fasern erlaubt es, die Transduktionsprozesse, die in den Bogengängen stattfinden, besser zu verstehen.

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SUBOCCIPITAL REMOVAL OF ACOUSTIC NEUROMAS

Results of 125 Operations

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Abstract The surgical results obtained in 125 patients with acoustic neuromas using the suboccipital approach are reported These results do not differ from those obtained in other neurosurgical materials, with the same distribution of tumour size, and the same surgical approach The material consists of 20 medium size tumours and 105 large However, the results regarding both mortality, postoperative facial paralysis as well as post operative condition in general are unsatisfactory A historical review of the development of the surgical treatment of acoustic neuromas is given and the necessity of a closer neurosurgical–otologic cooperation is stressed both with regard to diagnosis of the neuromas as well as the surgical treatment

previous publications we have dealt with diagnostic procedures and results among surgically confirmed acoustic neuroma We have evaluated the involvement of the facial, intermedius and trigeminal nerves at the diagnosis (Thomsen & Zilstorff, 1975), the audiological findings (Thomsen & Terkildsen, 1975) and recently the radiological and neuro-radiological findings in this group of patients (Thomsen et al . 1975) In the present paper we present the remaining diagnostic procedures employed on the same patients with surgically confirmed acoustic neuromas, as well as presenting the surgical findings, using the suboccipital approach The postoperative results as regards mortality, number of cases of facial nerve paralysis and general postoperative condition are presented and evaluated, partly in relation to the operative technique and partly to the tumour size and anatomical conditions

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HISTORICAL REMARKS

The first post-mortem description of an acoustic neuroma was probably that given by Sandifort in 1777 The first ever to correlate the clinical symptoms of an acoustic neuroma to the post-mortem findings was Leveque-Lasource in 1810 It was not until the end of the 19th century, however, that the localization of an acoustic neuroma was made on the basis of the clinical symptoms, prior to death Stevens (1879) was probably the first to describe this, even if it is generally accepted that Oppenheim (1890) was the first to make a correct topical diagnosis of a tumour in the cerebellopontine angle

Sir Charles Ballance (1907) is generally credited with the first successful removal of an acoustic neuroma The operation was performed on the 19th November, 1894, the patient lived 20 years subsequent to the operation The surgical approach to the cerebellopontine angle has varied considerably since this operation Krause described in 1903 the unilateral suboccipital craniectomy, while Panse in 1904 suggested that the most direct route to the cerebellopontine angle was through the labyrinth In 1905 Borchardt did

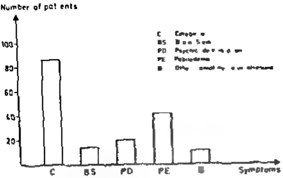

Fig 1 Symptoms other than V VII–VIII cranial nerve find ngs in 105 large acoustic neuromas

a combined operation where the translabyrinthine approach was extended posteriorly over the sigmoid sinus into the subocciput

In subsequent years several translabyrinthine operations for removal of acoustic neuromas (Kummel, 1909, Quix, 1911, 1912, Schmiegelow 1915, Zange 1915) have been tried However the translabyrinthine surgical technique disappeared from the arena, most likely due to the fact that Harvey Cushing introduced the bilateral suboccipital approach exposing the posterior fossa widely Cushing made a subtotal enucleation of the tumour and was able to reduce the operative mortality from 80% to somewhere between 10 and 20% Because of the problem of recurrence in some subtotal removal operations and the hazard of a second operation, Dandy in 1922 (Dandy 1925) reintroduced the total removal of the tumour via a unilateral suboccipital craniectomy as used around the turn of the century The suboccipital unilateral approach was hereafter pre-eminent and is still used by neurosurgeons up to the present day (Pool & Pava 1957, Rand & Kurze 1967)

Because of the constant high mortality and high frequency of postoperative facial nerve paralysis William House reintroduced the translabyrinthine surgical technique in 1961, the first results were published in Monograph I (1964)

Neuro-surgery was introduced as a specialty in Denmark by Eduard Busch, and both he and his successors have used the suboccipital approach Total removal has been endeavoured, but within certain limitations Some of the patients have therefore had only a subtotal enucleation It is our intention, in discussing the technique and the results, to examine the necessity of a closer neuro-surgical/oto surgical cooperation with respect to the possibility of improving the results

MATERIAL AND RESULTS

The material consists of 125 patients, operated at the neurosurgical department, Rigshospitalet in the years 1957–1972 All patients had acoustic neuromas The diagnostic findings and procedures concerning the V, VII, and VIII cranial nerves have been described in previous papers Likewise the neuroradiological findings have been presented In this material 105 large tumours and 20 medium size tumours were found Small tumours were not represented This distribution follows the classification of Pulec et al (1971)

The majority of the tumours were found in the 30–60-year age group Neuromas are rare before the age of 30 Women outnumber men by two to one 26% were examined with in the first year after onset of symptoms but 38% had a history longer than 5 years

85% gave hearing loss as the initial symptom, 10% gave tinnitus 4% started with vertigo and the last patient reported reduced facial sensibility as initial symptom In 7 patients bilateral tumours were found While neuromas are rare before the age of 30 4 of these 7 patients were younger than 30 All these 4 young patients had signs of von Recklinghausen's disease, the other 3 did not Six patients had been operated for acoustic neuroma before 1957 and are included in the material with a recurrent tumour operated in the period in question

The clinical symptoms, apart from V, VII, and VIII cranial nerve symptoms, are per definition excluded from the medium size

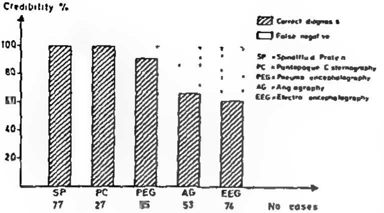

Fig 2 Credibility of diagnostic tests involving other than V–VII–VIII cranial nerve

tumours Fig 1 therefore contains only the 105 large tumours Cerebellar symptoms are by far the most frequent (83%). Choked discs were found in 42 patients (39%) indicating increased intracranial pressure Fourteen patients (12%) had brainstem symptoms, evaluated by long tract signs, while 15 patients (14%) had signs of psychic deterioration of varying degree 10% of the patients had symptoms from the other cranial nerves, the distribution of these symptoms is seen in Table I The X cranial nerve (nervus vagus) and the VI nerve (nervus abducens) are the most frequently involved, symptoms from [illegible]e other cranial nerves being present in only [illegible] patient each The patient with second [illegible] symptoms was blind, most probably [illegible] to increased intracranial pressure and choked discs Fig 2 shows the results of the examinations for spinal fluid protein, Pantopaque cisternography, pneumoencephalography, vertebral angiography and electroencephalography None of these examinations has been performed in all the patients The number of patients examined with each single test is seen in the figure There were no false negative findings by Pantopaque cisternography All patients had increased spinal fluid protein content For the medium size tumours the average was 88 mg%, varying between 55 and 182 mg%, while the large tumours had an average spinal fluid protein of 202 mg%, varying between 54 and 598 mg% Thus a significant difference is found, the large tumours having higher values than the medium size.

Operative technique

All the patients have been operated on by the unilateral, suboccipital approach to the cerebellopontine angle In the first part of the period the operation took place with the patient sitting in the Gardner chair, in the last half with the patient in a prone position, with the involved ear uppermost In the last third of the period the operation has been made with the aid of the operating microscope

A suboccipital "U" shaped, inferiorly based skin, muscle and periostial flap is elevated Occipital bone is removed from behind the sigmoid sinus Hyperventilation intravenous urea, and dependent trunk and lower extremities result in brain shrinkage The cere-

Table I *Symptoms from cranial nerves other than V–VII–VIII in 10 patients with acoustic neuromas*

Patient	Cranial nerve involved
M B	II
A S	III
T M	IV–IX–X–XI
F H	VI
J T	VI
S L	VI
J H	VI
N R	IX–X
S S	X
M E	X–XI
K A	XII

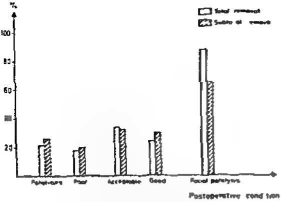

Fig 3 Influence of operative procedure on postoperative condition in 125 neuromas of all sizes

bellopontine angle and the VIII nerve neuroma will often be visible without forceful cerebellar retraction

In evaluating the patient's postoperative condition we have distinguished between good acceptable, and poor A patient in good condition has no symptoms except, possibly, facial paralysis The patient may even return to work again, bearing in mind however that many are elderly, and past the working age A patient in acceptable postoperative condition can help himself but has considerable neurological symptoms both from the other cranial nerves as well as suffering from cerebellar ataxia and difficulties in walking A patient in poor condition is either psychically deteriorated or physically unable to shift for himself

Fig 3 shows the distribution of mortality facial paralysis and postoperative condition in relation to the operative technique employed in 94 patients the tumour could be removed completely while 31 had only subtotal enucleation The primary mortality among the two groups equals 22% on average and the postoperative condition could be characterized as good in 27% poor in 18% while 33% had an acceptable condition There was no difference between total and subtotal removal 84% of the total material had postoperative facial paralysis—in 87% of

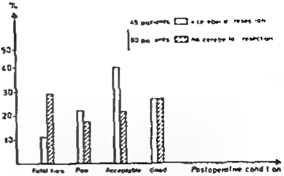

Fig 4 Influence of cerebellar resection on postoperative condition in 125 neuromas of all sizes

total removals, but in a lower percentage (61%) of subtotal removals

In 45 patients peroperative resection of a smaller or larger part of the cerebellum became necessary in one third to one fourth This resection cannot be connected with either the operative mortality or to the distribution of the postoperative conditions (Fig 4)

Fig 5 depicts the influence of surgically verified impression in the brain stem upon the postoperative condition While the impression apparently has no significant influence upon the mortality, it is clearly seen that a greater proportion of the group of patients with good postoperative condition lack brain stem impression Furthermore it is noted that anatomical impression in the brain stem does not necessarily imply clinical brain stem symptoms We found that only 8 out of 65 patients with anatomical impression in the

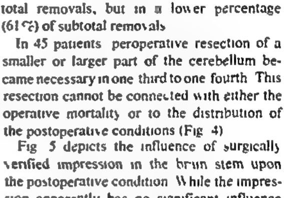

Fig 5 Influence of surgically confirmed brain stem impression on postoperative condition in 125 neuromas of all sizes

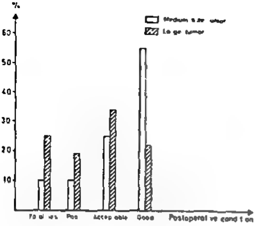

Fig 6 Influence of tumour size on postoperative condition in 125 patients with acoustic neuromas

brain stem had symptoms being indicative of this, while 5 patients had brain stem symptoms though no impression was found at operation

The material contains 20 medium size and 105 large tumours In Fig 6 their size has been related to frequency of mortality and postoperative condition The tendency in this figure is clear, since mortality is more often related to the large tumours, and the postoperative condition is significantly better percentwise among the medium size tumours than among the large ones

Sixty percent of the patients with medium size tumours developed postoperative facial paralysis, as against 70% of patients with large tumours The difference is not significant 50% respectively 60% of these patients underwent a facial-hypoglossus anastomosis These figures are seen in Fig 7, where we have furthermore tried to grade the function of the anastomosis at the last postoperative check With good function there is no disfiguration of the face at rest, but moderate changes during contraction of the mimic muscles In the patients with poor anastomosis function there is no detectable mimical function It is seen that about 15% of the patients in both groups have a well functioning anastomosis, while the majority had acceptable to poor function

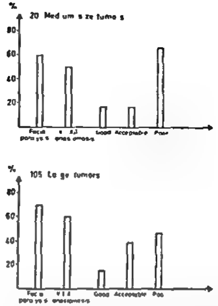

Fig 7 Frequency of postoperative facial paralysis facial hypoglossus anastomosis and function of the anastomosis in 20 medium size and 105 large acoustic neuromas

Fig 8 shows the influence of tumour size upon mortality rate and upon duration of hospital stay This implies only the stay in Rigshospitalet, some of the patients were transferred to other hospitals Among the patients with medium size tumours a primary mortality rate of 10% was found, 40% stayed

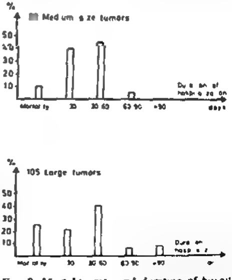

Fig 8 Mortality rate and duration of hospitalization in relation to tumour size in 20 medium size and 105 large acoustic neuromas

Table II *Cause of death in 28 operative fatalities, based on postmortem examinations Distribution of early/late deaths*

	Early <3 days	Late >3 days
Brain stem thrombosis	8	6
Pulmonary embolism	1	
Bleeding	1	3
Air embolism		1
Brain edema		1
Pneumonia		1
Encephalitis		2
Cerebellar malacia		1
Not established	2	1
	12	16

less than 30 days in hospital, 45% between 30 and 60 days only 5% between 60 and 90 days, no patient stayed longer than 90 days Among the 105 patients with large tumours the primary mortality rate was 25%, the hospital stay was generally longer, 8% of the patients stayed longer than 90 days

As mentioned, 28 patients of the 125 died in connection with the operation (22%) In Table II we have tried to distinguish between early (before 72 hours) deaths and late (from 3–30 days) deaths It is seen that in both groups the brain stem thrombosis and malacia are by far the dominant cause of death There was no difference between early and late death

Since the introduction of the operating microscope 39 operations for acoustic neuroma have been performed The mortality rate among these patients, with both medium size and large tumours combined has been lowered to 13%

DISCUSSION

As seen from the operative results both in this material and in publications of several other authors there is a significant connection between the size of an acoustic neuroma and the attainable results at operation A rapid development has taken place in the operative treatment of acoustic neuromas, especially since the introduction of the microsurgical technique and the use of other approaches to the cerebellopontine angle, in particular the translabyrinthine approach It seems, however, that in spite of refined diagnostic possibilities within oto neurology, audiology and neuro radiology, as if the tumours are as large as ever, in our material the large tumours are more numerous by far

To make a diagnosis of a disease it is a precondition that some symptoms must be present In the case of the acoustic neuroma it is clearly stated that the patients have symptoms fairly early in the history of the disease, viz prevailingly unilateral hearing loss and tinnitus The next precondition is that these symptoms prompt the patient to see his physician In our material a great number of patients have lived for years with their hearing loss and their tinnitus without seeing their physician A third precondition for the diagnosis of an acoustic neuroma will be that the physician connects the symptoms described by the patient with a possibility of a neuroma Some of the patients in our material have at some time during the history of the disease consulted their own physician without this resulting in a further consultation by an otologist If the patient finally is referred to a hospital department with qualified otologic service, the situation today is probably such that we will not overlook a neuroma

We have in our material a few cases where the patient was considered to be suffering from Meniere's disease These cases are from the earliest part of our period, and we believe that there is such a high degree of suspicion among otologists that the diagnosis is kept in mind, until disproven The paramount problem is to get the patients to examination at an early stage How this is obtained is impossible to say, there seems to be no patent solution to this problem There are, however, several materials published where the number of large tumours has been reduced considerably In these areas greater attention must be devoted to this problem both on the

part of the patient and of the referring physician

The results presented here probably do not diverge in any significant degree from the results obtained in other neuro-surgical departments, having similar patient materials and using the same surgical technique In evaluating the results, attention is drawn to the operative fatalities, the grade of sequelae after the operation, especially the frequency of facial paralysis, but also to the general postoperative condition

Until Cushing introduced his wide bilateral suboccipital approach and the subtotal enucleation, the operative mortality rate in acoustic neuroma cases was 75–80% Cushing was able to lower the mortality rate to about 20% This percentage lasted until the use of the operating microscope and the microsurgical technique was introduced In our material we had a mortality rate of 22% It is to be noted that after the introduction of the microsurgical technique this rate fell to 13%, and it is emphasized that the majority of our tumours must be classified as large

As is seen in Table II the majority of the fatalities are attributable to brain stem thrombosis or malacia In 1949 Atkinson described close relation between the anterior inferior rebellar artery and cerebellopontine angle umours He claimed, as the first, that the cause of the surgical catastrophes was manipulation or ligation of this artery, so vital for the blood supply to the brain stem If one is to compare the suboccipital with the translabyrinthine approach in this respect it can be stated that using the suboccipital approach this artery presents itself early in the dissection and must be pushed aside, manipulated, or even ligated whereas during the translabyrinthine operation the artery is situated between the tumour capsule and the brain stem, and in most cases it is not necessary to touch it

When considering the patient's postoperative condition, apart from the facial paralysis, it is especially the cerebellar ataxia which dominates the picture In some of our operations it was necessary to perform resection of varying magnitude of the cerebellum In the remaining cases one could avoid resection and merely retract the cerebellum However, the postoperative ataxia is found in the same frequency and severity in patients with only retraction of the cerebellum as in patients who had a part of the cerebellum resected during the operation It is therefore conceivable that it is the mere manipulation of the cerebellum which is the cause of the postoperative ataxia When using the translabyrinthine approach such a retraction is seldom necessary In few cases this retraction can be made extradurally The bone removal is carried posteriorly over the sigmoid sinus into the subocciput Access to the angle is obtained directly over the tumour and the dural covering over the cerebellum is not disturbed This procedure allows exposure of the inferior pole of the tumour and visualization of the important IX, X, and XI nerve complex Manipulation of the cerebellum is thereby minimized

The third serious complication to operations for acoustic neuromas is the occurrence of postoperative facial paralysis The surgeons of earlier times did not regard the presence of facial paralysis as serious complication The primary object was to save the patient's life Olivecrona (1941) was the first to report a series of operations where the facial nerve had been saved in a number of cases

The frequency of facial paralysis in our material is very high It should be noted that it is considerably lower, but still unacceptable, in the patients who had had subtotal removal only

In recent years several papers have been published clearly demonstrating a connection between tumour size and incidence of paralysis but especially a relation to the operative technique employed, as by the use of the translabyrinthine technique this incidence of facial paralysis has been considerably reduced The only safe way to identify the facial nerve is as indicated by House—quite lateral

in the internal acoustic meatus—and this is only possible via the translabyrinthine route In its course in the internal auditory canal and in the cerebellopontine angle the facial nerve is not enclosed in a proper nerve sheath, and is therefore often spread out over the tumourcapsule in an acoustic neuroma Only if the surgeon has with certainty identified the nerve has he a chance of separating capsule and nerve threads without lesion

In our material a facial hypoglossus anastomosis has been established in 60% of the paralysed cases The results of this anastomosis are seen in Fig 8, and these results can not be said to be satisfactory either The anastomosis is usually made 3–4 weeks after removal of the neuroma This is recommended by several authors, if one is convinced of having made a complete lesion of the facial nerve during the operation it is probably justifiable to make an early anastomosis But if the nerve is considered relatively intact, in spite of a postoperative paralysis it is maybe advisable to wait for quite some time, since Ojemann et al (1972) have reported cases of spontaneous recovery as late as one year after surgery for acoustic neuroma

Technically there is no doubt that the translabyrinthine approach is the most direct route to the internal acoustic meatus and the cerebellopontine angle The tumour is seen immediately when the posterior fossa dura is opened, and there is usually no reason to resect or manipulate the cerebellum The opponents of the translabyrinthine approach claim that the opening is too small However, this can easily be overcome by extending the approach extradurally over the sigmoid sinus into the subocciput

But the crucial point in the technique is to open the tumour capsule and remove the content from within Even the largest tumour can thus be reduced considerably and mobilized from the facial nerve and the brain stem in a reduced condition

Another critical point is the question about total vs subtotal removal of the tumour. Most authors recommend the total removal, and the critic of the translabyrinthine approach is based on an increased incidence of subtotal removals These figures, however, originate from the results of House's earlier operations as reported in Monograph I (1964), whereas the frequency of subtotal removals in the later publications has been reduced to about 10%, which is in agreement with the results of others Regarding the preservation of the facial nerve there is, via the translabyrinthine route, a precise bony landmark, i e Bill's bar (crista verticalis) in the lateral end of the internal auditory canal Only when this piece of bone is seen can one be sure identifying the facial nerve In dissections of the internal auditory canal through the posterior fossa there is no bony landmark to identify the nerve in the lateral aspect of the canal

Postoperative complications will occur with any approach There is probably a slightly increased tendency to cerebrospinal fluid leak from a translabyrinthine procedure, but after Montgomery et al (1966) introduced the use of abdominal fat as a plug in the operation cavity, instead of muscle this problem seems to have been reduced to a minimum

When in an otologic–neurosurgical cooperation one intends to introduce the translabyrinthine surgical technique it must be made quite clear that the otologists should not undertake the operation alone There must be a firm teamwork to ensure the safest treatment The procedure will most likely be that recommended by Yasargil & Fisch (1969), Ojemann et al (1972), Adams et al (1974), i e a primary translabyrinthine approach is performed doing all the necessary bone work and identifying the facial nerve by, for example inserting a piece of Silastic film between the tumour capsule and the nerve threads or by putting a nylon thread around the nerve If the tumour cannot be removed completely during this first procedure it is justified to perform a second op a week later, either by a suboc (Ojemann et al , 1972, Yas

or using a transtentorial approach (King, 1970, Adams et al , 1974) This procedure has been accepted by several neuro-surgeons

In Los Angeles, the Otologic Medical Group have operated by far the largest number of acoustic neuromas in history, about 800 patients They claim that a secondary suboccipital approach is unnecessary It would probably be impossible to aim that high to begin with

ZUSAMMENFASSUNG

Die Ergebnisse bei 125 Patienten deren Acusticusneurinom suboccipital operiert wurde werden vorgelegt das Krankengut umfaßt 20 mittelgroße und 105 große Tumoren Die Ergebnisse unterscheiden sich nicht von denen die in anderen neurochirurgischen Patientengruppen mit gleicher Tumorgrößenverteilung und Operationsmethode festgestellt wurden sie sind unbefriedigend sowohl hinsichtlich Mortalität und postoperativer Gesichtslahmungen wie betreffend dem allgemeinen postoperativen Zustand Es wird eine historische Ruckschau uber die Entwicklung der chirurgischen Behandlung von Acusticusneurinomen gegeben die Notwendigkeit einer engeren neurochirurgisch-otologischen Zusammenarbeit in der Diagnose sowie in der chirurgischen Behandlung der Neurinomen wird untersinchen

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DAMAGE TO REISSNER S MEMBRANE IN THE GUINEA-PIG COCHLEA FOLLOWING ACUTE ATOXYL INTOXICATION

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Abstract The ototoxic effects of the arsenic compound atoxyl has been earlier demonstrated by Anniko & Wersall (1975) in the stria vascularis of the guinea pig cochlea The aim of the present investigation was to study the morphological degeneration at the ultrastructural level after damaging the Reissner s membrane experimentally In acute atoxyl intoxication the first signs of degeneration appear after approximately 12 hours After 24 hours the membrane may be markedly degenerated Both the epithelial and mesothelial cells become vacuolized the mitochondria lose their internal structure and become swollen and lipid granules are found in the cytoplasm Mesothelial cells are damaged to a lesser extent than the epithelial cells in the same specimen

The ultrastructure of Reissner's membrane has been described in a number of publications (Hagiwara 1963, Duvall & Rhodes, 1967, Watanuki, 1968, Iurato & Taidelli, 1967, Duvall & Sutherland 1970) The membrane is situated between the spiral limbus and the vestibular crest of the spiral ligament, it is 2–3 nm thick, and consists of two layers Towards the endolymphatic space there is a simple squamous epithelium The surface facing the scala vestibuli consists of a layer of flat mesothelial cells A distinct basement membrane is interposed between the two cell layers (Iurato & Taidelli 1971) Reissner's membrane represents a portion of the membranous labyrinth where the amount of tissue separating endolymph and perilymph is minimal It may therefore play an important role in maintaining the difference in composition between the two fluids (Iurato, 1967)

The relative proportions of electrolytes in the endolymph are quite different from those in the perilymph (Smith et al , 1954, Citron et al , 1956, Rauch & Kostlin, 1958) Two hypotheses have been advanced to explain how this difference in composition is maintained

According to the "longitudinal flow" theory (Corti, 1851, Guild 1927, Lundquist, 1965) the endolymph is secreted in the stria vascularis, runs along the cochlear duct and is reabsorbed in the endolymphatic sac This theory, which is supported by Tasaki et al (1954) and Legouix (1962), also implies that Reissner's membrane is practically impermeable to sodium and potassium ions On the other hand, Naftalin & Harrison (1958), Lawrence et al (1961) and Rauch et al (1963) support the "radial flow" theory, i e the hypothesis that the endolymph is formed from perilymph by passing through Reissner's membrane According to this theory, the function of the stria vascularis is mainly absorptive

Compared to other tissues, the respiratory rate of Reissner's membrane is very high (Chou, 1963) Watanuki et al (...

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Table I *Administration of atoxyl to guinea pigs in the experimental group*

Animal no	Amount injected on each occasion (mg/kg)	Number of injections	Injection period (days)	Total amount injected (mg/kg)	Interval between last injection and sacrifice (days)	Preyer reflex
20	70	3	2	210	1	–
52	70	2	1/4	140	1/4	–
53	100	1		100	1	(+)
54	70	2	1/4	140	1/4	(+)
55	140	1		140	1	–
63	140	1		140	1/2	–

a higher turnover of nucleic acid (DNA) in the epithelial cells on the inner side of Reissner's membrane than in the mesothelial cells on the outer side of it Plester (1963) reported reduced protein synthesis in Reissner's membrane after kanamycin administration Misrahy et al (1962) reported increased permeability after kanamycin treatment Kaneko et al (1970) suggest that after kanamycin administration Reissner's membrane may lose its ability to maintain the normal chemical composition of the perilymph and endolymph

Tracers have been introduced into the scala vestibuli by many investigators (Tonndorf et al 1962, Rudert 1969 Duvall & Quick 1969, von Ilberg 1968, Rauch 1966) There appears a selective passage of some dyes (meth blue toluidine blue and ferritin) through membrane while others (methyl blue anus green) do not pass through it (von Bekesy 1953 Duvall & Tonndorf 1962 Hinojosa 1971)

Nowak (1974) reported altered permeability of Reissner's membrane following streptomycin administration

The aim of the present study was to investigate the effect on Reissner's membrane of atoxyl (Pro Gen® Sodium) an arsenic compound (sodium arsenilate) with known ototoxic properties (Anniko & Wersall 1975)

MATERIALS AND METHODS

Nine healthy, young guinea pigs with a normal Preyer's reflex, weighing around 250–350 g were used for the experiment. Six animals were injected with sodium arsanilate (atoxyl) The control group consisted of 3 healthy, untreated guinea pigs

Each animal was injected subcutaneously with a 2% solution of atoxyl in sterile water over a certain period of time The amount of atoxyl injected on each occasion varied between 70 and 140 mg per kg body weight The total dose ranged from 100–210 mg per kg body weight The period of administration varied between 6 hours and 2 days and the survival time before decapitation between 6 hours and 1 day after the last injection (Table I) The specimens were treated according to standard methods for light and electron microscopy of osmium tetroxide fixed specimens (Anniko & Wersall, 1975)

RESULTS

Structural alterations were observed as early as 12 hours after atoxyl administration After 24–48 hours marked degeneration of Reissner's membrane was observed

Administration of 70 mg of atoxyl per kg body weight twice within 48 hours and sacrificing the guinea pig 24 hours after the last injection resulted in very severe damage to the membrane in all coils there being no apparent difference between the turns Changes in all coils were observed when 70 mg of atoxyl per kg body weight was administered twice within 6 hours and the animal was sacrificed 12 hours after the first injection The Reissner's membrane in the basal coils in these animals was however less severely damaged

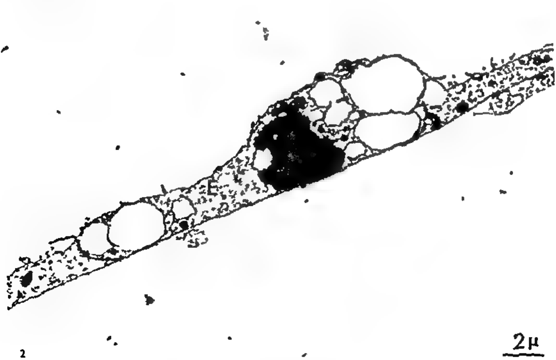

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Fig. 1 The position of Reissner's membrane is changed during the degeneration and it becomes depressed over the organ of Corti as the damage proceeds. Light microscopy ×700

Fig. 2 Electron micrograph (EM). Degenerating endothelial (*E*) cell with massive vacuolization

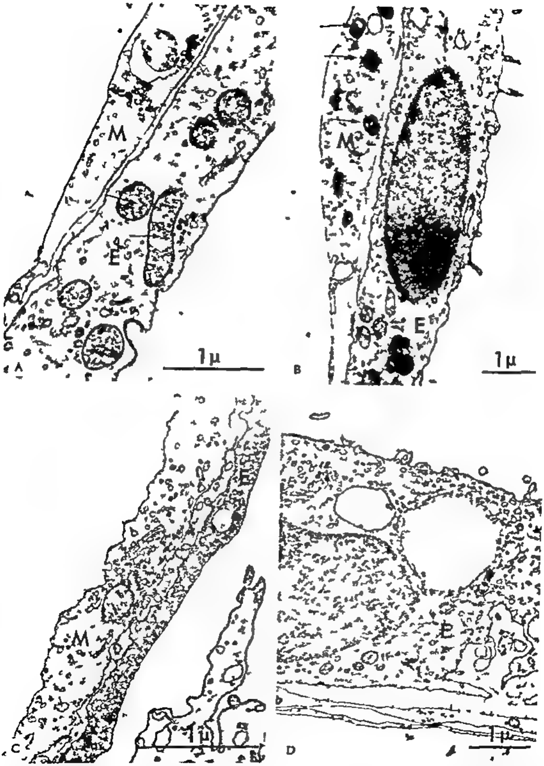

Fig 3 (A) EM Endothel al (*E*) and mesothel al (*M*) cells with mitochondria showing fragmentat on of the r internal structure (*arro s*) (B) EM Accumulations of electron dense material in the cytoplasm (m tochondria?) of endothel al (*E*) and mesothel al (*M*) cells (*arrows*) (C) EM D fferent at on of the degenerat ve pattern of he two cell types in Reissner s membrane the ele on-dense appearance of the endothel al (*E*) cell cytopla m can be seen (D) EM L po d l ke accumulations in the cytoplasm of an endothel al (*E*) cell

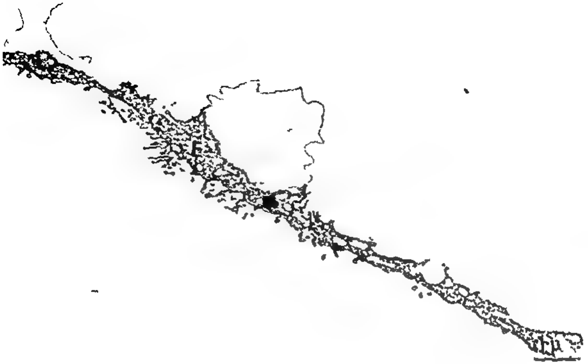

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Fig 4 (A) EM A "dark" endothelial cell (E) with a high degree of distortion The remains of the mesothelial cell reveal a large vacuole facing the perilymph (B) EM Accumulations of very electron-dense material in the cytoplasm of a "dark" endothelial (E) cell

than the rest of the cochlea Animals which received 100 mg of atoxyl per kg body weight 24 hours before sacrifice showed only minimal degeneration of the Reissner's membrane restricted to the apical coils The same changes were observed when 140 mg of atoxyl per kg body weight was administered and the animals were sacrificed after the same time interval The labyrinth from animals sacrificed 12 hours after such treatment showed no morphological changes

The position of Reissner's membrane alters depending on the degree of intoxication In some animals with early degeneration of the membrane the whole membrane was seen to bulge out towards the scala vestibuli (hydrops labyrinthine) In severe atoxyl intoxication the membrane become depressed and lies over the tectorial membrane and the Hensen cells but seldom touches the stria vascularis (Fig 1)

All the cells within Reissner's membrane were affected, the most severe degeneration being regularly found in the apical coils Lesions occur both in the insertion of the membrane at the stria vascularis and in the area of the insertion at the spiral limbus

squamous epithelial cells

squamous epithelium cells which normally contains a moderate number of vesicles and pinocytotic vesicles, showed different degrees of vacuolization which was so widespread in some specimens that almost the whole cell was filled with vacuoles and the degenerating cell organelles are pressed towards the cell periphery (Fig 2) The vacuolization becomes most widespread in the area around the nucleous However, vacuoles of considerable size may also be observed in the region of the cell junction between two squamous epithelium cells

The normal cell appearance with microvilli, tubules of the endoplasmic reticula a small Golgi apparatus a few microtubules and filaments and pinocytotic vesicles, is lost rather early during the degeneration process Reductions in the numbers of microvilli and pinocytotic vesicles are the first signs of damage The mitochondria swell and the cristae mitochondriales become fragmented (Fig 3 A) In severely damaged cells the mitochondria contain varying amounts of electron dense, osmiophilic material and this may even fill the mitochondrion completely (Fig 3 B) Lipoid like granules as large as 10–50 mitochondria are observed in the cytoplasm at all stages of degeneration (Fig 3 D) Some severely damaged squamous epithelium cells throughout the cytoplasm of the cell have an electron dense appearance (Fig 3 C) The cells are distorted but they still take part in the lining of Reissner's membrane separating the two fluid compartments from each other (Fig 4 A, B) The free surface of the epithelial cell layer towards the endolymph is uneven However, free ribosomes, damaged mitochondria, fat granules and also accumulations of even more electron dense material are also observed in these cells The vacuolization is poor

The cell nucleous is resistant to degeneration and is the last cell component to disintegrate It becomes involved in the final stage of damage by fragmentation of the chromatin and rupture of the nuclear membrane

The mesothelial cells

The degeneration of the mesothelial cells follows a similar course to that of the epithelial cells The cells become vacuolized very few pinocytotic vesicles are observed and the internal structure of the mitochondria is fragmented but the mitochondria have fewer accumulations of electron dense material than those in the epithelial cells Few fat granules are seen and they are not of the same size as those in the epithelial cells Mesothelial cells with an electron dense appearance similar to that of the cytoplasm of the whole cell have not been found The mesothelial cells are always less degenerated than the squamous epithelial cells in the same specimen Mesothelial cells adjacent to the scala vesti

built frequently contained rather large vacuoles on the cell membrane facing the perilymph

The degeneration of Reissner's membrane starts in the apical part and it must undergo rather severe changes before its position changes The electron dense squamous epithelium cells were only found in animals where the Reissner's membrane had changed from its normal position

DISCUSSION

The present investigation reveals that atoxyl has a damaging effect on Reissner's membrane in the guinea pig In the initial phase of degeneration no pathological changes are observed in the light microscope, while the electron microscope shows morphological alterations

Kaneko et al (1970) reported that after kanamycin administration the epithelial cells in Reissner's membrane showed vacuolization and condensation of cytoplasmic granules They classified the cells into two types according to their cytoplasmic appearance—light and dark cells The light cells showed vacuolization of the cytoplasm and little change in most of the cell organelles On the other hand, the density of mitochondria and cell granules in the dark cells was increased Both cell types showed decreased numbers of pinocytotic vesicles

The present results demonstrate the existence of some cells with comparatively dense cytoplasm However although the cell organelles in these "dark" cells are more electron dense than normal they take part in the degenerative process

It has been reported from electron microscopic studies that the epithelial cells of Reissner s membrane contain many vesicles which may be involved in micropinocytosis A well developed Golgi complex is found in the cytoplasm and many microvilli are observed on the endolymphatic surface (Hagiwara 1963 Iurato 1967 Duvall & Rhodes, 1967) These findings suggest a high degree of metabolic activity

In guinea pigs made deaf by the administration of kanamycin, micropinocytotic vesicles were very rarely seen in the epithelial cells of Reissner's membrane Therefore, it is unlikely that active transport by the pinocytotic system is functional in these cells (Kaneko et al , 1970)

The two pathways from the scala vestibuli to the scala media through Reissner's membrane via the intercellular junction and via pinocytosis have been demonstrated by Duvall & Quick (1969) and Duvall & Sutherland (1970)

Misrahy et al (1962) reported increased permeability after kanamycin administration, hypoxia and acoustic overstimulation Nowak (1974) showed vacuolization, increased formation of lysosomes, lipid granules and an increased number of free ribosomes after dihydrostreptomycin administration Fragmentation of the zonula occludents and desmosomes was also reported Schatzle (1972) proposed that the lipid granules were formed by damage of the lysosomes, resulting in the accumulation of lysosomal lipids

An increased number of dark bodies is frequently observed in the epithelial cells of Reissner's membrane in atoxyl intoxicated guinea pigs However, it is difficult to establish their true identity without histochemical studies

The mesothelial cells adjacent to the scala vestibuli frequently exhibited protrusions of the cell membrane facing the perilymph However, this only occurred when the total amount of atoxyl was very large and the specimen was investigated more than 24 hours after beginning the treatment The cell organelles of the mesothelial cells took part in the degeneration in the same way as those in the epithelial cells but never showed such severe damage in the same specimen The dark epithelial cells appear more distorted than the light cells, but the degeneration proceeds similarly, except that the vesiculation is minimal No intermediate stages between the dark and light cells were observed

The changes in the position of Reissner's membrane after atoxyl administration have earlier been described by Anniko & Wersall (1975) These changes indicate that strial damage causes a blockage of the endolymph circulation, resulting in an alteration in the position of the membrane However, blockage of the transport activity in the Reissner's membrane itself might also influence its position provided passage of fluid and electrolytes by pinocytotic activity is taking place in the normal membrane Atoxyl treatment thus results in damage to Reissner's membrane which successively degenerates and loses its normal fluid transport function

ZUSAMMENFASSUNG

Uber die ototoxische Wirkung des arsenhaltigen Atoxyls auf die Feinstruktur der Stria vascularis wurde bereits berichtet (Anniko & Wersall 1975) In dieser Studie werden die degenerativen Veranderungen der Reißner Membran nach Atoxyl Applikation beschrieben Schon 12 Stunden nach Atoxyl-Gabe konnen die ersten Degenerationszeichen der Reißner Membran feinstrukturell nachweisbar sein Nach 24 Stunden ist die Schadigung gewöhnlich sehr ausgepragt Epithelzellen und Mesothelzellen sind stark vakuolisiert ihre Mitochondrien sind geschwollen und zeigen Bruche ihrer Cristae im Cytoplasma sieht man Lipidgranula Die Epithelzellen scheinen starker geschadigt als die Mesothelzellen

ACKNOWLEDGEMENT

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EFFECT OF PHLORIZIN ON GLUCOSE TRANSPORT IN THE INNER EAR

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Abstract ^{14}C-glucose was used as a tracer to study the effect of phlorizin on glucose transport into perilymph and endolymph The transport was found to be lowered in phlorizin-injected animals compared with that in normal controls This inhibitory effect corresponding to a phlorizin-sensitive part of glucose transport was greater in the perilymphatic system than in the endolymphatic system Pathways which are not affected by phlorizin are considered to exist in both compartments of perilymph and endolymph

Glucose is essential for the maintenance of auditory function in sensory cells of the cochlea Glycolysis in the hair cells is considered to provide biochemical energy for the generation of cochlear microphonics Another function of glucose in inner ear fluids is concerned with a role in the regulation of osmotic pressure It is generally held that electrolytes protein and glucose are main chemical components which contribute to the osmotic pressure in biological fluid However as only a few studies have been published on the glucose transport into inner ear fluids (Komaki 1972 Makimoto & Silverstein 1974) further investigation is warranted

According to Danielli (1954) glucose permeability through the cell membrane can be classified into following three types passive diffusion facilitated diffusion and active transport Glucose transport is usually active in tubular cells of the kidney though glucose is transported by facilitated diffusion in the red cell membrane (Wilbrandt & Rosenberg 1951) This difference indicates that the mechanism of glucose transport differs according to the organ in question

It is known that phlorizin a glucoside with the chemical structure as shown in Fig 1, exerts specific inhibition on the active transport of glucose in intestinal wall and tubular cells of the kidney It is considered that phlorizin inhibits active transport of glucose through the cell membrane because of its high affinity to the carrier (Lotspeich & Woronkow, 1962) and that this affinity is 100 to 10000 times greater than that of glucose (Chan & Lotspeich, 1962)

In the present study an attempt was made to clarify the characteristics of glucose transport in the inner ear by investigating the effect of phlorizin on transport of ^{14}C glucose into inner ear fluids of guinea pig

MATERIALS AND METHODS

Forty three normal guinea pigs and seventeen phlorizin injected guinea pigs weighing about 300 g were anesthetized with Nembutal (35 mg/100 g) given intraperitoneally

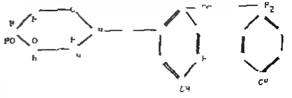

Fig 1 Chemical structure of phlorizin

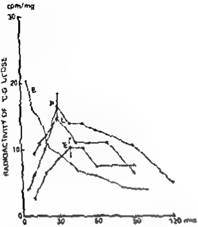

Fig 2 Curve of radioactivity in perilymph endolymph cerebrospinal fluid and blood in control guinea pigs *P* perilymph, *E* endolymph *L* cerebrospinal fluid *B* blood

Samples of perilymph, endolymph, cerebrospinal fluid, and blood were collected in the time course after a venous infusion of ^{14}C-glucose solution Perilymph was collected by the following procedure the tip of a glass microcapillary mounted on a micromanipulator was introduced through the round window membrane into the scala tympani with the aid of an operating microscope To obtain endolymph, basilar membrane was exposed by removing the round window edge Endolymph was collected by directing the microcapillary tip into the basilar membrane Cerebrospinal fluid was collected from the cisterna magna All samples contaminated with blood were discarded

^{14}C-glucose with specific activity of 5 0 mCi/mmole was dissolved in distilled water to make a ^{14}C-glucose solution (0 01 mCi/ml) At a dose of 0 3 ml/300 g, this ^{14}C glucose solution was infused into the femoral vein In the phlorizin experiment, the phlorizin solution (1 mg/ml) was given 10 min before the infusion of ^{14}C-glucose, intraperitoneally at a dose of 1 ml/300 g

In each animal, perilymph and endolymph were taken respectively from both ears at two different points in the time course, likewise cerebrospinal fluid and blood were collected respectively at three to four different points Each sample which was obtained in this way, provided one sample for radioactivity counting

Blood samples (0 01 ml) which were collected by a venesection in the cervical region, were diluted 1 1000 with distilled water. For deproteinization 0 01 ml of 10% metaphosphoric acid was added, then supernatant was obtained by centrifugation The weights of the samples of inner ear fluids and the cerebrospinal fluid were measured using a microbalance, and the samples were then dried and fixed at a room temperature of 24°C

The radioactivity of ^{14}C-glucose in samples was measured using a gas flow counter Q gas (He-gas) was employed as the flow gas A scaler connected with 2π flow counter (Shimazu Seisakusho Ltd D-55, B-301) was used for determining the radioactivity The background noise was first counted for 10 min, then the samples were counted for the next 10 min Counts per minute (cpm) were calculated as follows

$$\text{cpm} = \frac{\text{10 min sample count} - \text{10 min background noise count}}{10}$$

RESULTS

Normal guinea pigs

The radioactivities of perilymph, endolymph, cerebrospinal fluid and blood are shown in

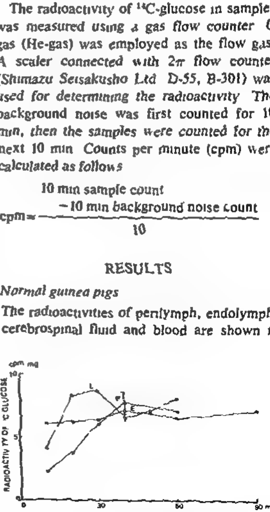

Fig 3 Curve of radioactivity in perilymph endolymph and cerebrospinal fluid in phlorizin-injected guinea pigs *P* perilymph *E* endolymph *L* cerebrospinal fluid

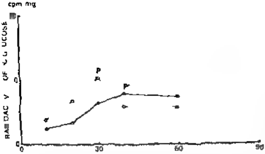

Fig. 6. Curve of phlorizin-independent glucose transport in perilymph compared with that of phlorizin-dependent glucose transport. P: phlorizin-independent, and P_e: phlorizin-dependent glucose transport in perilymph.

COMMENTS

The present results revealed that in normal guinea pigs glucose is transported into perilymph and cerebrospinal fluid at a higher rate than it is into endolymph. The curve of ¹⁴C glucose rises simultaneously in perilymph and in cerebrospinal fluid and these two curves appear to be similar in their pattern of time course. However differences were observed between these two radioactivity curves, e.g., the rising curve to peak is sharper and the peak value higher in perilymph. If the entry of glucose into perilymph were to depend only on cerebrospinal fluid, the peak of radioactivity in perilymph might be expected to follow the peak in cerebrospinal fluid. This evidence of a higher radioactivity in perilymph suggests different characteristics of glucose transport for perilymph.

Graf & Poretti (1950) reported that the radioactivity of ²⁴Na appeared almost simultaneously in perilymph and in cerebrospinal fluid after subcutaneous injection of radioactive sodium solution. The relationship of the curve between the perilymph and cerebrospinal fluid was found to be in good coincidence with the findings in the aforementioned.

Shortly after the intravenous infusion of ¹⁴C glucose, the radioactivity in endolymph increased and reached a peak, then decreased relatively slowly. This may indicate that although glucose is transported into endolymph at a higher rate, the transported glucose remains in the endolymph for a longer time. The curve of ¹⁴C glucose in perilymph was higher than that obtained in endolymph, and the difference between two curves in peak value was 6.11 cpm, which is significant ($p<0.05$, $t=2.815$). These facts point to the existence of a strong barrier to the glucose transport between the perilymphatic and the endolymphatic system.

Phlorizin is known as a competitive inhibitor of the active transport of glucose through the cell membrane (Alvarada & Crane, 1962). The curve of ¹⁴C glucose radioactivity in perilymph and that in endolymph shifted down by administration of phlorizin as shown in Figs. 3–5. One can assume from these findings that the glucose transport into perilymph and endolymph is inhibited by phlorizin. Since a greater fall of ¹⁴C glucose radioactivity was exhibited in perilymph than in endolymph, it appears that the inhibitory effect of phlorizin on glucose transport is greater in the perilymphatic system than it is in the endolymphatic system.

Glucose transport is considered to consist of two courses in the brush border of the renal tubule. The one is inhibited by phlorizin and the other is sensitive to the aerobic metabolism (Wilson, 1964). In the intestinal wall 10% of the glucose is transported by oxida

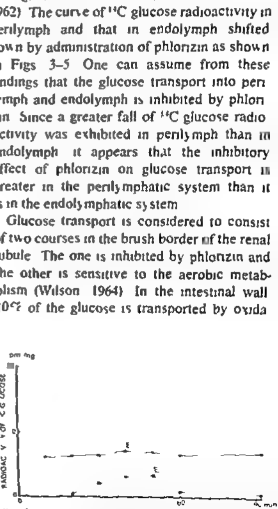

Fig. 7. Curve of phlorizin-independent glucose transport in endolymph compared with that of phlorizin-dependent glucose transport. E: phlorizin-independent and E_e: phlorizin-dependent glucose transport in endolymph.

tive phosphorylation, and the remaining 90% by other processes (Landau & Wilson 1959)

When phlorizin is given to guinea pigs at the same dose as in the present study (1 ml/300 g with 1 mg/ml of phlorizin solution) the concentration of phlorizin in blood is calculated to be approximately 2×10^{-4} M/l This concentration of phlorizin is sufficient for complete inhibition of glucose transport through membrane (Alvarada & Crane 1962, Schultz & Zalusky, 1964) Thus it is conceivable that the glucose transport which was not affected by phlorizin in this experiment is achieved through phlorizin independent pathways

The curve of phlorizin independent glucose transport in perilymph was compared with that of phlorizin dependent glucose transport in Fig 6 The phlorizin dependent curve (P_0) was drawn by plotting the values which were derived by subtracting the values of radioactivity in perilymph of phlorizin injected guinea pigs from those of control animals ($P-P'=P_0$) Here P and P' represent the radioactivity for control and phlorizin injected guinea pigs respectively

The curve of phlorizin independent glucose transport in endolymph was compared with that of phlorizin dependent glucose transport in Fig 7 The phlorizin dependent curve (E_0) was obtained in the same way as in the case of perilymph ($E-E'=E_0$) Here E and E' represent the radioactivity for normal and phlorizin injected guinea pigs respectively

From the present study it is clear that glucose transport into perilymph and endolymph depends partly on the pathway which is inhibited by phlorizin Consequently it is also concluded that there may be pathways other than the phlorizin dependent one, both in the perilymphatic and in the endolymphatic system

Chemical identifications of the ^{14}C glucose in various fluids of guinea pig were not performed in this study According to our previous experiment (Komaki 1972) employing the scanning of radioactivity for paper chromatogram, the peak of radioactivity on paper chromatogram was proved to correspond to the R_f of D glucose (0 18) in each sample

ZUSAMMENFASSUNG

Mittels ^{14}C Glukose als Tracersubstanz wird die Wirkung von *Phlorizin* auf den Glukosetransport in das endo- und perilymphatische System untersucht Der Transport bei *Tieren denen Phlorizin injiziert wurde* ist ein deutig niedriger als bei einer Kontrollgruppe Diese inhibitorische Wirkung die einem phlorizinsensitiven Teil des Glukosetransportes entspricht ist im perilymphatischen System großer als im endolymphatischen Im Bereich des peri und endolymphatischen Systems scheinen Nebenwege zu bestehen die nicht von Phlorizin beeinflußt werden

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THE ROLE OF THYROXINE IN THE DIFFERENTIATION OF THE ORGAN OF CORTI

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Abstract The developmental bas s of the association of congenital deafness with hypothyroidism observed in several human syndromes was earlier invest gated by experiments on mice The offspring of females with chem cally induced hypothyroid sm were found to have malformat ons of the organ of Corti showing that thy rox ne played a role n the d fferentiat on of th s organ The present study was undertaken to identify the s te of act on of the hormone in the develop ng organ It appears to be the ep thel um of the inner spiral sulcus

The association of congenital loss of hearing with dysfunction of the thyroid gland has been observed in both hereditary and non hereditary syndromes (Trotter 1960) The hereditary syndromes have been more fully investigated although owing to the high degree of variability of clinical features and the obvious difficulty of ascertaining whether any two similar disorders in man are indeed caused by genes at the same locus it is not clear how many distinct genetic entities are involved (Fraser 1965 McKusick 1971) Those hereditary cases in which the thyroid dysfunction takes the form of a goitre are classified as Pendred s syndrome after Vaughan Pendred (1896) who first described it and are generally treated as a single genetic entity

As to the non hereditary syndromes the association of deaf mutism with endemic cretinism and goitre is well known What is not so widely recognized is the fact that in congenitally deaf persons without a manifest goitre the incidence of thyroid malfunction is appreciably higher than in the general population Baschieri et al (1963) tested 42 congenital deaf mutes without goitre for the ability of potassium perchlorate to discharge radio iodine and found that in 21 of them the discharge occurred showing that there was a block in the organic binding of iodine in the thyroid Similar findings have been reported for students of Clarke School for the Deaf at Northampton Mass USA (Brown 1969) It was also found that there were more persons with the level of protein bound iodine below 4 μg/100 ml among the relatives of these students than expected indicating that some of the apparently non hereditary syndromes are probably hereditary

In order to elucidate the relationship between hypothyroidism and deafness female mice were given the thyroid inhibitor 6-*n* propyl 2 thiouracil (PTU) in the drinking water and the inner ears of their offspring examined (Deol 1973) The controls consisted of the offspring of untreated females as well as of those that had been given sodium L thyroxine in addition to PTU It was found that treatment with PTU if begun before the 15th day of pregnancy (gestation period about 21 days) produced striking and consistent abnormalities in the organ of Corti The organ w

Fig. 1. Normal 3 days basal half turn ×255

Fig. 2. Normal 6 days basal half turn ×255

Fig. 3. Normal 14 days basal half turn ×255

Fig. 4. Normal 29 days basal half turn ×255

Fig. 5. Normal 29 days apical half turn ×255

mal in the offspring of untreated females and of those that had been given thyroxine in addition to PTU at any time up to 9 days after birth. These abnormalities were independent of the effects of PTU on general growth, for the addition of the hormone eliminated the abnormalities without improving the overall growth during the critical period. The present investigation was concerned with the development of these abnormalities of the organ of Corti, the aim being to identify the site of action of the hormone.

MATERIALS AND METHODS

This report is based on examination of the inner ear in 32 mice with abnormalities of the organ of Corti produced by treating the mother with PTU and 20 mice with normal organ of Corti born to untreated mothers, all from the inbred strain C57BL/Gr. They ranged in age from newborn to 60 days old. The majority in both groups fell in the range of newborn to 15 days old at 3 day intervals, this being the crucial period from the present point of view. The interval of 3 days between successive age groups was not too long because the fine differentiation of the organ of Corti begins in the basal part of the cochlear duct and pro-[illegible]

The animals were fixed in Witmaack's fluid, decalcified in 1% nitric acid, and the auditory part of the head was embedded in celloidin and paraffin (for details see Deol, 1970). Serial sections were cut at 10 μ and stained with haematoxylin and orange G.

OBSERVATIONS

The cochlear duct in the mouse makes a little over one and a half turns and for convenience can be divided into basal, middle and apical half turns. In what follows attention will be focused mainly on the tectorial membrane and the inner spiral sulcus, for it is here that the effects of PTU-treatment are most evident.

Normal mice

In newborn normal mice all major elements of the organ of Corti could be identified, although they were far from resembling their mature form. The epithelium of the inner sulcus consisted of columnar cells, and was in contact with the tectorial membrane in all places. The membrane was thin and fairly uniform throughout. At 3 days the differentiation of the hair cells and the supporting elements was only slightly more advanced, and there was no important change in the sulcus epithelium, but the tectorial membrane had grown much thicker (Fig. 1). At [illegible] days the hair cells were considerably more advanced, and the tunnel had made its appearance. In the basal half turn the cells of the sulcus epithelium had begun to lose their columnar shape in the inner corner to become flatter, and the tectorial membrane had thinned down appreciably (Fig. 2). As a result a space had appeared between the epithelium and the membrane, the beginning of the sulcus itself. In the middle half turn the space was much smaller and the membrane was still thick, while in the apical half turn the space had yet to appear. At 9 days the hair cells and the supporting elements had advanced still further, and the tunnel was well-developed. The sulcus epithelium had become flat except in the vicinity of the inner hair cells. The development of the sulcus was nearly complete, but the tectorial membrane was still a little thicker than in the adult.

At 12 days the organ of Corti had reached the adult form in all essential respects, and this was even clearer in 14 and 15 days old animals (Fig. 3). Functional maturity obviously goes in step with histological maturity, for normal mice begin to hear at about 13 days. Further changes in the organ were confined to the organization of the supporting ce

Fig 6 PTU treated 3 days basal half turn ×255

Fig 7 PTU treated [illegible] days basal half turn ×255

Fig 8 PTU treated 9 days basal half turn ×255

Fig 9 PTU treated 14 days basal half turn ×255

Fig 10 PTU treated 25 days basal half turn ×255

Fig 11 PTU treated 55 days basal half turn ×?55

Fig 12 PTU treated 35 days apical half turn ×255

Fig 13 PTU treated 55 days apical half turn ×255

the outer part The adult organ in the basal and apical half turns is shown in Figs 4 and 5 respectively

PTU treated mice

Up to the age of 3 days the organ of Corti in PTU treated mice appeared to be similar to that in the normal (Fig 6) Differences were first observed at 6 days and were virtually confined to the region of the inner sulcus The epithelium of the sulcus was still columnar and in contact with the tectorial membrane throughout There was no thinning of the membrane in any part In the basal half turn the membrane was distorted in shape as well as in the arrangement of its fibrils (Fig 7) The distortion was not observed in the middle and apical half turns and since these regions represent earlier stages of development the distortion had probably made its appearance not much before 6 days At 9 days the whole organ of Corti was different from that in normal animals (Fig 8) So far as the hair cells and the supporting elements were concerned this difference amounted to little more than arrested development but the tectorial membrane was unequivocally malformed In the basal half turn the distortion of its fibrillar arrangement had become much more marked and it had acquired a sharp hump (Fig 8) These abnormalities were less striking in the middle half turn and barely detectable

in the apical one The epithelium of the sulcus was columnar throughout and in contact with the tectorial membrane At 12 days the hair cells and the supporting elements still appeared to be arrested at the 6-day stage, there being no tunnel yet, while the abnormalities of the tectorial membrane had become even more pronounced In the basal half turn its outer end had become withdrawn from the hair cells and was now resting on the epithelium of the sulcus, forming a kind of 'foot' The body of the membrane had become more severely humped and its internal structure further disorganized These abnormalities were milder in the middle half turn, and only slight in the apical one There was no sign as yet of the flattening of the sulcus epithelium, and naturally none of the sulcus space either In 14 and 15 days old animals the hair cells and the supporting elements had made some progress, and although greatly retarded they were otherwise fairly normal (Fig 9) The outer end of the tectorial membrane had withdrawn still further from the hair cells, as if its 'foot' was slowly moving along the sulcus epithelium towards the vestibular lip The epithelium itself had begun to grow flat in the inner corner in the basal half turn giving se to a small space under the membrane

rther differentiation of the organ of Corti characterized by a very slow maturation the hair cells and the supporting elements which eventually reached a fairly normal adult form in most parts of the cochlear duct (Figs 10–13) The epithelium of the sulcus also gradually became flat But the malformations of the tectorial membrane grew in severity, the 'foot' eventually making contact with the vestibular lip in the basal half turn and most of the middle one, and the fibrillar arrangement becoming extremely disorganized, leading to the formation of spaces within the membrane (Fig 11) In the apical half turn, however, it retained its immature thick form, with only a moderate degree of distortion, although it was nowhere in contact with the hair cells (Figs 12, 13)

DISCUSSION

The abnormalities of the hair cells and the supporting elements observed in PTU-treated mice are comparatively minor They can be viewed as a reflection of the general slowing down of the differentiation of the organ of Corti, for these cells eventually attained, or did so very nearly, the normal form in most of the cochlear duct The abnormalities of the tectorial membrane, on the other hand are quite different in character Even at 6 days, when they were first observed, they could not be treated purely as an aspect of the slowing down of the differentiation of the organ of Corti, for the fibrillar arrangement of the membrane showed distinct signs of distortion From then onwards at all stages the membrane in PTU-treated mice showed clear differences from the earlier stages in the normal, and it never even approached the normal form in the adult The tectorial membrane would then appear to be the element specifically affected by the treatment

The tectorial membrane is a non cellular structure formed by the epithelium of the inner spiral sulcus, except in the region that is attached to the limbus, where it is formed by the epithelium of that part, which is continuous with that of the sulcus (Weibel 1957) As the membrane is composed largely of protein (Iurato 1967) its constituents are presumably actually secreted by the epithelium, because the machinery for protein synthesis occurs in the cytoplasm The treatment with PTU may then be seen as affecting the epithelium of the inner sulcus the source of the membrane

It appears that it may not be the formation of the membrane that is at fault since there is no difference between normal and treated animals at 3 days, but its maturation the process of its thinning down to adult dimensions without the disruption of its connection with the hair cells This process in normal mice is accompanied by radical changes in the cytology of the sulcus epithelium In other

words the membrane and the epithelium mature simultaneously as the epithelial cells lose their columnar shape and withdraw from the membrane, the latter becomes thinner In view of the non-cellular nature of the membrane, and the facts that it is produced by the epithelium and the maturation of the two is synchronous, one may reasonably assume that the maturation of the membrane is also a function of the epithelium

The failure of the tectorial membrane to mature normally in PTU treated mice may, therefore be seen as an abnormality of the sulcus epithelium It is well established that thyroxine can play a role in the control of protein synthesis (Tata, 1971) and it is possible that the epithelium fails to form the substance or substances necessary for the maturation of the membrane in the absence of the hormone The specific site of action of thyroxine in the developing inner ear would then be the epithelium of the inner sulcus and the critical stage would be the period during which the maturation of the tectorial membrane takes place This is supported by the observations that the abnormalities of the membrane are eliminated if thyroxine is added to the drinking water containing PTU at 6 days their severity is greatly mitigated if it is added at 9 days but they are unaffected if it is added at 12 days (Deol 1973)

ZUSAMMENFASSUNG

Die Entwicklungsgrundlage der Verbindung zwischen angeborener Taubheit und Hyposchilddrusenfunktion die in verschiedenen menschlichen Syndromen beobachtet werden kann wurde in fruheren Experimenten mit Mausen untersucht In der Nachkommenschaft von Weibchen mit chemisch induzierter Hyposchilddrusenfunktion fand man Mißbildungen im Cortischen Organ die zeigten daß Tyroxin eine Rolle in der Differenzierung dieses Organes spielt Die gegenwartige Untersuchung wurde ausgefuhrt um den Wirkungsort des Hormones im entwickelnden Organ zu identifizieren Er scheint das Epithel des Sulcus spiralis internus zu sein

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PHYSIOLOGICAL AND CLINICAL ASPECTS OF THE REHABILITATION OF TOTAL DEAFNESS BY IMPLANTATION OF MULTIPLE INTRACOCHLEAR ELECTRODES

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Abstract Many instances of total deafness are due to destruction of the organ of Corti contrasting with the partial or complete preservation of the function of the cochlear nerve In such cases it is possible to restore some hearing by stimulating electrically the fibres of the cochlear nerve with the help of implanted electrodes By means of several fenestrations it is possible to construct electrically insulated compartments in the scala tympani of the cochlea and to implant eight electrodes This procedure allows discrimination of sound frequencies because the electrical stimulation of each electrode gives a sound sensation which depends on the electrode stimulated This enabled us to elaborate a map of frequencies of the human cochlea and to make some physiological observations and achieve clinical results in even cases of total bilateral deafness

This technique proposed as early as 1957 by Djourno & Eyries for the saccular nerve then tried by Simmons (1966) for the cochlear nerve was recently reintroduced with the use of a single electrode and developed simultaneously by Merzenich et al in San Francisco (1973) and by House & Urban (1973) in Los Angeles

However appreciable the auditive gain thus offered to these patients may be it nevertheless remains restricted by the poverty of frequency information In order to improve these results the implantation of several electrodes was deemed to be indispensable by all participants in the Symposium held on this subject in San Francisco in June 1973

Since then we have tackled this problem personally This enabled us to gather some physiological observations and results that appeared to be worth reporting as they may be helpful in understanding the difficulties and requirements when rehabilitating by implantation of multiple electrodes

The justification for this work must be stated first Animal experiments would not have brought us much information concerning the intelligibility of received messages On the other hand in such psychologically fragile patients as the totally deaf no risk of failure could be assumed The first trials had therefore to be conducted in patients with unilateral deafness it was however impossible to perform such trials with a purely experimental purpose in patients with isolated deafness On the other hand when this deafness was associated with facial paralysis implantation could be proposed and performed at the same time as the operation aiming at correcting this paralysis The results obtained under these conditions in 3 patients (Chouard & MacLeod 1973) subsequently enabled us to operate on our first patients with total and bilateral deafness

PHYSIOLOGICAL CONSIDERATIONS

Since the work by von Békésy (1960) the sound waves that may be reduced to a

complex set of sinusoidal waves whose frequencies and intensities vary with time are known to be transmitted to the fluids in the scala vestibuli and scala tympani as hydraulic longitudinal vibrations These are converted into transverse vibrations at the level of the ductus cochlearis and reach their peak intensity in an area whose topography depends directly on the frequency high pitches are located at the base of the cochlea and low pitches at its apex All along the canalis spiralis modioli 40 000 fibres of the cochlear nerve are spread in the form of a fan reproducing this frequency topography that ranges over approximately ten octaves in humans Each nerve fibre therefore has a frequency specificity that is peculiar to itself but this elective specificity does not prevent it from being more or less sensitive to many other close frequencies more readily to low than to high frequencies

A sound stimulus normally results in an electrophysiological activity whose topography within the nerve depends on its frequency Frequency information is wholly contained in this topographic distribution that is in fact to be found also in the diencephalon and cortex However at low frequencies below 300 Hz the spikes of the nerve fibres arise as bursts whose rhythm reproduces the frequency of the stimulus Above this frequency the bursts merge the rhythm gets lost and is no longer recognizable subjectively Up to ca 1 500 Hz in spite of this physiological non recognition this rhythm could be identified by a computer Beyond frequencies of 4 000–5 000 Hz the distribution of the spikes becomes wholly random (Rose et al 1967) When the intensity of the stimulus increases the number of spikes also increases so that irrespective of the frequency the number of spikes is proportional to this intensity This increase in the number of spikes when the intensity of a pure tone increases is first achieved by an acceleration of the small contingent of fibres electively sensitive to this frequency But the refractory period of the nerve fibre prevents this acceleration from proceeding beyond 100 spikes per second

Any further increase in intensity results in bringing into play adjacent fibres theoretically specific of somewhat different frequencies The increase in the intensity of a tone thus results in a subjective alteration of its purity

The electric stimulation of the cochlear nerve can be performed with sinusoidal signals only the negative half of which is in fact physiologically useful But it is more economical to use square waves It is difficult to stimulate directly and distinctly the fibres within the nerve trunk into which they get gathered and condensed On the contrary it is much simpler to make use of their spreading along the lamina spiralis of the cochlea by implanting the electrodes at the level of the scalae This stimulation can be either total or partial in both cases it is transmitted through the cochlear fluids

The total stimulation of the cochlea can be either direct or indirect The indirect stimulation is obtained by placing the electrode in contact with the round window in the tympanic cavity This quite simple operation must be performed under local anaesthesia for the sound sensation perceived to be identifiable it is in this way that those patients with total deafness who are likely to benefit by implantation can be identified

The total stimulation of the cochlea can be performed directly by chronically implanting the electrode into the scala tympani This procedure was recently followed by the two American schools with either a bipolar (Merzenich et al 1973) or a unipolar electrode (House & Urban 1973) and we also followed it at the beginning of our work with a unipolar electrode

SURGICAL AND ELECTROPHYSIOLOGICAL PROCEDURES

We used Teflon-coated microelectrode wire of 10% iridium 90% platinum bare diameter 0 005 inch

In our first patients, only one electrode, then two and five were implanted in the cochlea

The broad frequency sensitivity of the nerve fibres for a given sound intensity, contrasting with their selectivity in the vicinity of the threshold, enables implantation of a restricted number of electrodes, provided that they are distributed along conversation frequencies We do not know yet what will be the necessary and sufficient number, but the results obtained with electronic reconstruction of speech allow one to think that it will be less than 12

For that reason we now in cases of total bilateral deafness implant 6 to 8 electrodes in different sites in the inner ear

Each electrode was included in the cochlea through a fenestration of the scala tympani An electrically insulated compartment was made in the scala for each electrode by means of little pieces of silastic Each electrode was fixed to the bone around the fenestration with methylmetacrylate

Two surgical approaches are necessary for this implantation (*a*) the middle ear approach with resection of the bone of the external auditory meatus and conservation of the skin d ear-drum This provides good access to basal turn, second turn and apex (*b*) The ans temporal supra petrosal approach allows access to the highest internal portion of the first turn of the cochlea, between the geniculate ganglion and the internal auditory meatus This approach is indispensable because the conversational frequencies lie in this region

This operation was performed in 7 cases of total bilateral deafness Subjective responses were studied with each electrode receiving square wave signals of variable voltage and frequency

In addition to these implantations we performed an indirect stimulation in 45 subjects by placing an electrode in contact with the round window We constructed an apparatus that divides the sound message by mean of filters, into eight groups of frequencies at octave intervals, from 125 to 8000 Hz The sinusoidal signal received in each channel is converted into impulses whose frequency varies with their intensity According to their position in the cochlea, the electrodes are connected with the corresponding channels of the apparatus through Teflon plugs, ensuring the transmission between the internal and external media For this multichannel device, we have not yet tried the wireless percutaneous transmission of messages

RESULTS

Electrophysiological findings

We performed total stimulation of the cochlea It was most frequently an indirect stimulation via the transtympanic approach, but in two cases it was a direct stimulation by the way of a single electrode In these cases of total stimulation, the fibres of the cochlear nerve are concerned as a whole, for the electric impulse diffuses through the fluids to the whole canalis spiralis modioli, regardless of the location of the electrode The result is a white noise in which high sounds are somewhat predominant, probably because the greater width of the scalae at the beginning of the cochlea favours the diffusion of the current in this area at the expense of the narrowed areas at the apex of the cochlea This white noise is perceived irrespective of the frequency of the electric stimulus and only the physiologically perceptible rhythms present in this stimulus, i e those whose frequency is less than 300 Hz are perceived Thus, when the frequency of the signal is varied below this figure a false impression of tonal discrimination can be felt analogous for instance to the noise of the explosions of a motorcycle engine, first idle, then accelerating, until giving the impression of a continuous sound when a frequency of 300 Hz is reached But the analogy is not complete In contrast to the sound shocks of an engine, the electric stimuli, when they further ac-

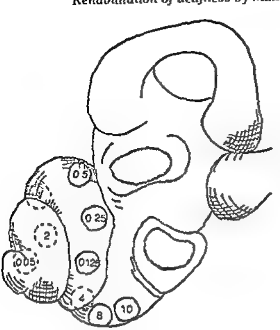

Fig 1 Map of the topographical repartition of frequencies in the human cochlea

celerate, merely induce within all nerve fibres an increased intensity of the white noise obtained by fusion

We made three observations in our patients with a single electrode implanted chronically as well as in patients stimulated in the area of the round window But the exploration of these acoustic phenomena was possible only because we had to deal with patients with unilateral total deafness capable of comparing the perceived sound by the healthy ear These facts account very well for the results reported both by House & Urban (1973) and by Merzenich et al (1973) The latter authors stress moreover the non differentiation of frequencies above a limit around 600–700 Hz though we cannot explain satisfactorily the difference from the 300 Hz we obtained

If the cochlea is divided into several electrically insulated compartments it becomes possible to perform a local stimulation involving only one compartment For a given stimulus whose frequency exceeds 300 Hz applied in succession to each electrode the sound perceived depends on the topography of the electrode We could thus plot a frequency map of the cochlea which had never been done until now in man (Fig 1) It corresponds fairly well to what could be extrapolated from animal investigations By stimulating separately one of the electrodes with a stimulus whose frequency is less than 300 Hz, the same phenomena are obtained of frequency variation, acceleration, then fusion as with total stimulation, except that the sounds resulting from the fusion, instead of being a white noise, approximate in this case a pure tone corresponding to the frequency topography of the stimulating electrode In fact, at the frequency of 1 Hz, the sound heard is comparable to either a gong or hand bell stroke according to the electrode concerned More precisely, when an electrode lying close to the apex cochleae is stimulated beginning with a frequency under 30 Hz, the gradual acceleration of the stimulus gives a sound whose pitch increases up to the frequency of 300 Hz in the vicinity of which the fusion of each sound results suddenly in a new much deeper sound, a rumbling in the case taken as an example

It is important to emphasize that conversation frequencies lie on the hidden face of the cochlea which can only be approached by the suprapetrosal route

Another difficulty is worth pointing out During this electric stimulation of the cochlear fibres, there is a very narrow margin between the liminal and discomfort thresholds In voltage the latter is approximately twice as high as the former The enormous dynamic compression effected by the tympano-ossicular system and above all by the cochlear fluids and Corti s organ is easily conceived According to our observations this particular trouble is well eliminated if constant level impulses whose frequency increases with stimulus intensity are used as stimuli

CLINICAL RESULTS

However fragmentary they may be these results are encouraging A twofold aspect

diagnostic and therapeutic, must be distinguished in them.

(a) Diagnostic aspect

The indirect total stimulation of the cochlea through the round window always gave us positive results in 45 cases of total and bilateral deafness, except for one patient operated upon for auditory neurinoma, which is perfectly understandable. It is worth pointing out that this test showed that implantation was worth performing in 8 cases of meningitis (meningococcal, mumps, or of undetermined origin), 6 cases of labyrinth destruction due to petrosal fracture, 12 cases of congenital deafness, 5 cases of childhood deafness, 4 cases of bilateral labyrinthitis with cholesteatoma of undetermined origin, 4 cases of otosclerosis extended to the labyrinth and 1 case of severe long-standing Meniere's syndrome. However small the number of tests performed may be, the majority of the cases of total deafness may be thought to retain a more or less partial cochlear function and therefore to be worth implanting.

Children's pusillanimity does not allow us to perform this test under local anaesthesia. We divided the examination into two stages: positioning of the electrode under general anaesthesia. Then, on the following day, stimulation in the family environment, in an alert, reassured child.

We were able to record the evoked auditory potentials caused by electric stimulation in our implanted patients. It is probable that the application of this technique in very young infants would facilitate early detection in those amenable to implantation.

(b) Therapeutic aspect

During the last 6 months, we have implanted 7 patients with total deafness, namely: (i) a 56-year-old female who had been deaf for 2 years following meningitis, (ii) a 42-year-old male who had been deaf for 39 years with no precise cause, (iii) a 20 year-old male who had been deaf for 10 years, deafness had developed gradually, over 2 years, with no precise cause; (iv) a 70-year-old female who had been deaf for 8 years as a result of bilateral chronic labyrinthitis, (v) a 46-year-old female who had been deaf for 38 years after childhood meningitis; (vi) a 43-year-old female who had been deaf for 5 years after meningitis, (vii) a 26-year-old male who had been deaf for 16 years as a result of bilateral petrosal fracture.

As early as the day after the electrode positioning, the discrimination of frequencies and the understanding of a few words are possible. But these immediate performances imply lip reading, the more so when the patient has been deaf for a longer time. Nevertheless, these performances are always superior to those obtained when a single electrode is stimulated. Melodic recognition is variable from patient to patient, it may reach almost 100% in some of them.

After about one month, the intelligibility of learned list words remain poor. One may estimate that about 50% of ordinary words are understood by the patient without lip-reading. It is indeed a question of simple and everyday words which help the deaf person to understand the other words in the sentence.

In our 3 patients whose long standing deafness had resulted in voice changes, the voice improved dramatically as early as the first weeks.

These results are fragmentary and their study is still incomplete. Many problems remain to be solved, notably as regards the miniaturization of the percutaneous transmission of the message: many unknown elements persist, in particular as to the tolerability of implanted materials after several years. But it is reasonable to hope that these difficulties will be overcome in the near future.

ZUSAMMENFASSUNG

In vielen Fallen ist die Ursache totaler Taubheit eine Destruktion des Cortischen Organs, wahrend dagegen die

Funktion des Hornervs partiell oder vollstandig erhalten ist In solchen Fallen ist es moglich durch elektrische Stimulation der Fasern des Hornervs mit Hilfe von ein operierten Elektroden wieder ein gewisses Horen zuruck zuerhalten Mittels einer Reihe von Fensterungen wurden elektrisch voneinander getrennte Abschnitte in der Scala tympani der Schnecke angelegt und dort acht Elektroden einoperiert Dieses Verfahren ermoglicht eine Diskrimi nation von Tonfrequenzen da die elektrische Stimulation der verschiedenen Elektroden eine Schallempfindung hervorruft die jeweils von der stimulierten Elektrode abhangt Auf diese Weise konnte eine Frequenzlage karte der menschlichen Schnecke aufgenommen werden und des weiteren eine Reihe von physiologischen Beob achtungen und klinischen Resultaten an sieben Fallen mit totaler b lateraler Taubheit gewonnen werden

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PRESSURE DEPENDENT VARIATION IN VOLUME OF MUCOSAL LINING OF THE MIDDLE EAR

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Abstract A method is described for studying pressure dependent variation in the volume of the mucosa of the middle ear Studies were performed at different pressures in the middle ear as well as at different ambient pressures It was found that the pressure dependent volumetric changes of the mucosa were the same whether the pressure in the middle ear was changed directly by altering the intratympanic or indirectly by altering the ambient pressure With the method described it is also possible to determine the middle ear volume without artefacts due to the middle ear mucosa The volume pressure relationship of the middle ear mucosa varied from 0 6 to 1 7 $\mu l/cm\ H_2O$ and linearly with the volume of the middle ear Comparisons between determinations of the middle ear volume with and without consideration of the mucosal compliance showed differences especially in small middle ears The effect on the volume of the mucosa caused by variation of posture was also studied The physiological middle ear pressure depends on the functional state of the Eustachian tube the middle ear volume the tympanic membrane and the middle ear mucosa Thus knowledge of the mucosa compliance is important for calculating middle ear pressure as well as for determining the volume of the air filled middle ear space The method might also prove a useful tool in the elucidation of the vascular bed both in health and in disease as well as the reaction of the mucosal vessels to drugs

List of symbols

P_{atm}	atmospheric pressure (in the laboratory)
P_m	pressure in the middle ear
P_{ch}	chamber pressure
V_m	volume of the air filled middle ear
V_{tm}	change in volume of middle ear due to tympanic membrane movements on increase or decrease of pressure applied called volume displacement in the text This displacement of the ear drum is given relative to its neutral position outwards (+) inwards (−)
V_{muc}	volume of the mucous membrane lining the middle ear
V_m	air flow through resistor of the experimental system i e system connected to patient on compression or decompression of the gases in the middle ear
V_{syst}	air flow through resistor on compression or decompression of gases within the recording systems and within the external ear canal
V_{muc}	air flow through resistor on compression or decompression of gas in the middle ear due to variation of the volume of the middle ear mucosa
P_{cuff}	pressure gradient over the rubber disc in the external ear canal
C_{muc}	compliance of the middle ear mucosa $\Delta V_{muc}/\Delta P_m$
Δ	before a symbol indicates a change in a variable Pressure is expressed in cm H_2O volume in μl or ml and air flow in $\mu l/sec$
P_m and P_{ch}	are relative to atmospheric pressure in the laboratory (considered constant during an experiment)

The bony walls of the middle ear are rigid but the tympanic membrane and the mucosal lining however are to a certain degree compliant to pressure variations (ΔV_{tm} and ΔV_{muc}, Fig 1) Hence the middle ear varies in volume with the movements of the tympanic membrane (ΔV_{tm}) and the volumetric changes of the middle ear mucosa The variation in the

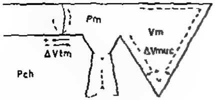


Fig 1 Middle ear model with its variables For explanation of symbols see text

volume of the middle ear mucosa (ΔV_{muc}) is caused by variation in the amount of blood in the vessels of the mucosa, which in turn varies with the intratympanic pressure (P_m) and vascular tone

It was thought that investigation of the pressure dependent variation of the volume of the mucosa might shed new light on the properties of the vascular bed in health and in disease and its reaction to drugs as well as on the widely assumed effect of the state of the mucosa on tubal function Knowledge of the variation of the volume of the mucosa with pressure is necessary for determining the volume of the middle ear (V_m) both directly through a perforated ear drum (Flisberg et al , 1963, Riu et al 1966) as well as indirectly across an intact ear drum (Ingelstedt et al , 1967, Elner et al 1971)

Ingelstedt et al (1967), who studied the effect of variation of venous pressure on the volume of the middle ear mucosa, found a linear correlation between ΔV_{muc} and the change in pressure within the range of 0 to +10 cm H_2O, ΔV_{muc} was, on the average, 0 42 $\mu l/cm\ H_2O$ They assumed that the variation in volume would be the same also when the variation in P_m was brought about by a change in the pressure acting directly on the mucosa This assumption is important in the determination of V_m However, variation of P_m affects all structures in the middle ear, including all blood and lymphatic vessels, while variation of the venous pressure affects mainly the veins and probably the capillaries

The aim of this investigation was threefold, viz

(1) to develop a method for quantitative determination of ΔV_{muc} on exposure of the mucosa to different pressures,
(2) to elucidate the effect of variation of posture on ΔV_{muc},
(3) to devise a method for measuring the volume of the middle ear without interference by ΔV_{muc}

MATERIAL

The clinical material consisted of 5 subjects (4 women, 1 man) with traumatic perforation of the drum Inspection with an operating microscope showed that the middle ear was completely dry and that the mucosa appeared normal in all 5 subjects

METHOD AND EQUIPMENT

The experiments were performed in a pressure chamber in which it was possible to change any overpressure between 0 and 50 cm H_2O to a corresponding underpressure within 25 seconds or vice versa

A polyethylene catheter running through a rubber disc was inserted airtight into the inner bony part of the external ear canal The other end of the catheter was attached to a flowmeter (Fig 2) This system here called the test system records flow of air caused by the flow of air into and out of the middle ear, V_m, and by compression or decompression of the gas within the recording system and within the external ear canal The latter flow, V_{syst}, can be regarded as an artefact occurring on change of the pressure in the system This artefact V_{syst}, is determined and eliminated by a 'reference system i e a system with properties identical with those of the test-system and connected to a sham external ear canal with a volume of 0 3 ml From the signal of the test-system i e $V_m + V_{syst}$ the signal from the reference system V_{syst} is subtracted by an electronic circuit providing a signal corresponding to V_m The latter signal is integrated to obtain a signal corresponding to volumes entering or leaving the middle ear, ΔV_m The change in volume was recorded on

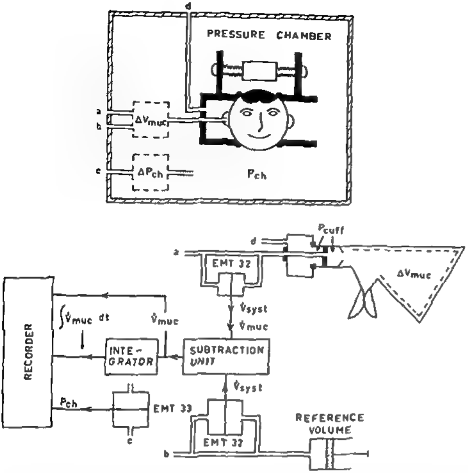


Fig. 2. Outline of the equipment used for the recordings. The subjects were placed in a pressure chamber with a cap fixed airtight around the external ear. The pressure in the cap was the same as in the middle ear in order to avoid a pressure gradient across the rubber disc (P_{cuff}). In the figure *a–d* are connected to atmospheric pressure which corresponds to the experimental set up for determination of ΔV_{muc} according to procedure A. When performing experiments according to procedure C–E *a–d* were connected to chamber pressure or to an external pressure device. A middle ear model is seen (*lower right*) connected to the test system and the reference volume to the reference system. Both systems consist of a flow meter, i.e. a resistor (pneumotachograph) and a pressure transducer (EMT 32, Siemens Elema). For more details see text.

an X-Y recorder via a tape recorder as a function of the change in pressure prevailing in the chamber.

In experiments when the chamber pressure was changed while the pressure in the middle ear was constant, the signals $\dot{V}_m$ and ΔV_m reflect changes in mucosal volume, i.e. $\dot{V}_{muc}$ and ΔV_{muc}. In experiments with changes in the middle ear pressure and constant chamber pressure, ΔV_m reflects any ΔV_{muc} inclusive volume compression or decompression of the middle ear gas.

The use of two identical systems is of fundamental importance as different errors caused by varying temperature and pressure affecting transducers and connecting lines are eliminated by subtraction of one signal from the other.

In order to avoid sliding of the rubber disc in the external ear canal owing to a difference between the pressure in the ear and that in the chamber, caps were fixed over the subject's external ears. This prevented pressure gradients across the rubber disc (P_{cuff}, Fig. 2). For a more detailed description of the recording system, see Elner et al. (1971).

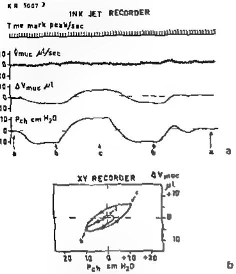


Fig 3 (*a*) Recordings of $\dot{V}_{muc}$ ΔV_{muc} and P_{ch} according to procedure A (*b*) Recordings of corresponding volume/pressure relationship (X Y recorder)

PROCEDURES AND RESULTS

A *Determination of ΔV_{muc} by changing the ambient pressure (P_{ch})*

The middle ear and the caps were connected to the atmospheric pressure Lowering of the chamber pressure (P_{ch}) to say −10 cm H O caused a relative overpressure in the middle ear and a decrease in volume of the middle ear mucosa (Fig 3*a* *b*) When the chamber pressure was then changed from −10 to +10 cm H O the volume of the middle ear mucosa increased owing to the relative underpressure in the middle ear ΔV_{muc} was continuously recorded during the pressure change as well as for another 10 sec during constant chamber pressure At the end of each experiment it was checked that the volume signal of the middle ear mucosa had returned to the original value The curve for the relation between volume and pressure was ellipsoid indicating a delay between the volumetric change in relation to the chamber pressure (Figs 3 and 4) The wider the pressure variation the longer the delay A purely static condition was never reached while the chamber pressure was constant (10 sec) A slow volume variation was observed even after 10 sec The variations in volume seem to be of two kinds one fast and quantitatively dominant and one slow (slow type)

Fig 5 shows static volume pressure diagrams of the middle ear mucosa in all the patients The values were measured from the end points of each loop (Fig 4) When the pressure changes were small (≤10 cm H_2O) the covariation of the volume was linear When the pressure changes were larger (>10 cm H O) the correlation between ΔV_{muc} and P_{ch} was nonlinear The compliance of the middle ear mucosa C_{muc} i e $\Delta V_{muc}/\Delta P_{ch}$ was de-

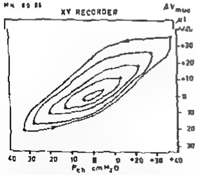


Fig 4 Loops demonstrating the volume/pressure relationship at different pressure changes according to procedure A The endpoints of each loop were used when determining the diagram presented in Fig 5

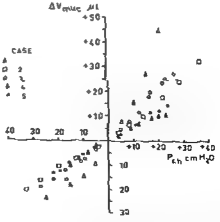


Fig 5 Diagram demonstrating the volume/pressure relationship according to procedure A A linear relation was found with a pressure range of < ±10 cm H O while a more varying relation was seen when the chamber pressure changes exceeded ±10 cm H O

Table I Compliance of the middle ear mucosa C_{mue} determined according to procedure A and calculated within a pressure range of $\leq \pm 10$ cm H_2O

V_m according to procedure E without correction for ΔV_{mue} are compared to data with such a correction The comparisons show that considerable errors may occur as concerns small middle ears if no correction for ΔV_{mue} is made

Case	C_{mue} (µl/cm H_2O)	V_m according to procedure D (ml)	V_m according to procedure E (ml) (not corrected for ΔV_{mue})	V_m according to procedure E (ml) (corrected for ΔV_{mue})
1 △	1 70	21 0	24 0	22 0
2 □	0 65	1 7	2 7	2 2
3 ○	0 97	10 0	11 0	10 0
4 ×	0 56	1 ?	1 8	1 2
5 ●	0 61	3 0	3 5	2 9

termined within the pressure range of ± 10 cm H_2O (Table I) The results showed a close relationship between C_{mue} and V_m (Fig 6)

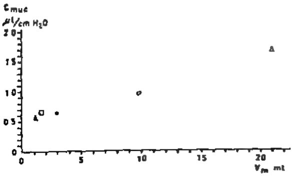
B *Determination of ΔV_{mue} with change in posture*

The flow of gas in and out of the middle ear caused by changes in volume of the middle ear mucosa with posture was recorded The subjects were seated in a heart chair whose back could be tilted from almost vertical (85°) down to a horizontal position (0°)

Fig 7 gives the individual ΔV_{mue} values on change of posture in relation to V_m The results indicate that ΔV_{mue} is not directly proportional to V_m

C *Passive opening of Eustachian tube in association with a fast and slow increase in middle ear pressure*

The experiments were performed in order to find out whether pressure-dependent changes in the volume of the middle ear mucosa influences the passive opening of the Eustachian tubes The caps the middle ear and the reference system (Fig 2*a b d*) were exposed to the atmospheric pressure The chamber pressure was lowered and ΔV_{mue} was recorded The sudden opening of the Eustachian tube appeared clearly on the recording This opening of the tube was not caused by any active manoeuvre by the subject but by a relative overpressure in the middle ear high



symbols as in Fig 5

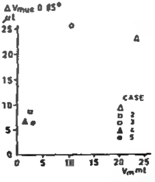


Fig 7 Results from experiments according to procedure B i e the variation of ΔV_{mue} caused by the change of body posture in relation to the air filled middle ear volumes

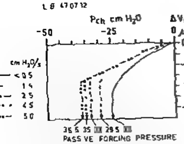


Fig 8 Example of recordings of ΔV_{muc} during slow and fast pressure decreases in the pressure chamber starting at atmospheric pressure and continuing until the Eustachian tube was forced open passively (procedure C) For more details see text

enough to force the tube open. This pressure is called "passive forcing pressure" Experiments with a varying rate of change in the chamber pressure were performed Fig 8 gives an example of the results from such experiments Similar results were obtained in experiments on the other patients When the middle ear pressure was increased slowly it caused a larger reduction in the volume of the mucosa, and the passive forcing pressure was lower than when the pressure changes were faster

D *Determination of volume of middle ear (V_m) without influence of ΔV_{muc}*

By connecting *a*, *b* and *d* (Fig 2) to the chamber pressure, any change in chamber pressure caused an immediate and equal pressure change in the caps and the middle ear The caps served no purpose in these experiments and the pressure in the middle ear was equal to the ambient pressure (P_{ch}) throughout the experiment

An increase in chamber pressure caused an air-flow into the middle ear owing solely to compression of the gas (ΔV_m) From the recording of ΔV_m and ΔP_m, V_m was calculated according to Boyle's law

$$V_m = \frac{\Delta V_m \,(P_{atm} - 47 \text{ mmHg})}{\Delta P_m} \qquad (1)$$

in which P_{atm} is the barometric pressure and 47 the pressure of water vapour at 37°C

The values recorded for V_m (Table I) were not influenced by errors due to variation in volume of the middle ear mucosa or by slipping of the rubber disc in the external ear canal The linear relationship (closed loop) between ΔV_m and ΔP_{ch} was expected as the change in volume (ΔV_m) was due solely to compression of the gas (Fig 9)

E *Determination of V_m and ΔV_{muc}*

By connecting *a*, *b* and *d* to an external pressure device it was possible to alter the pressure change in the middle ear, the caps and the reference system (Fig 2) The ambient pressure (chamber pressure) was constant during the experiments (atmospheric pressure) The pressure chamber served no purpose in these experiments

Changes in pressure cause volumetric displacements in the recording system partly because of compression or expansion of the middle ear gas and partly because of variation in the volume of the middle ear mucosa

When V_m was calculated according to eq 1, i e ignoring the variation in volume of the middle ear mucosa, the calculated figures were too high (Table I) When calculating V_m after elimination of the individual V_{muc}-factor, i e according to eq II, the values were not

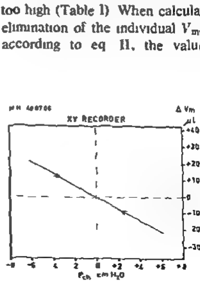


Fig 9 Recording of ΔV_m according to procedure D (see text)

significantly different from the largely correct values found by procedure D

$$V_m = \frac{(\Delta V_m - \Delta P_m \times C_{muc})\,(P_{a\,m} - 47\text{ mmHg})}{\Delta P_m} \qquad (2)$$

DISCUSSION

The present material was small partly because of difficulties in finding suitable test subjects i e healthy middle ears with a traumatic perforation partly because of the technical difficulties Ingelstedt et al (1967) showed that *the volume of the middle ear mucosa increased* by on the average 0 42 μl/cm H_2O of the venous pressure In the present investigation corresponding values i e the compliance of the mucosa were about 60% higher than those found by Ingelstedt et al (1967) even when the patient with an unusually large middle ear was excluded

Since the data found in the two investigations showed no overlapping the difference found appears to be a true one The main part of the difference must apparently be due to the differences in the methods used for altering e pressure In the present investigation the anges in pressure affected not only the veins nd capillaries as in the investigation done by Ingelstedt et al (1967) but also the lymph vessels the Eustachian tube and the round *and oval windows Volumetric changes of* slow type might be responsible for part of *the differences seen and might be due to dis*placement of intercellular fluid between the mucosa and the surrounding structures The pressure applied were maintained for a shorter time in the earlier investigation However slow type changes in volume in the present investigation were responsible for only about 10% of the total volumetric change which is only a minor fraction of the total difference (60%) What concerns volumetric changes contributed from the round and oval windows these must be insignificant (Ivarsson & Pedersen 1975)

The tests with a slow and a rapid increase in the pressure of the middle ear (procedure C Fig 8) showed that the slow pressure increase was associated with a large volume change of the mucosa *resulting in opening of the tube* at relatively low pressures These low forcing pressures mainly reflect decongestion of the mucosa but fatigue of the tissues operating the closure of the Eustachian tube might also be a contributory factor

The tests with change in posture showed somewhat larger volumetric changes than could be expected if the variation was caused by the increased venous pressure alone The *actual change of posture has been found to* cause a venous pressure increase at the level of the middle ear (bulbus vena jugularis) of about 10 cm H O (Jonson & Rundcrantz 1969) If the recorded volumetric mucosa changes was caused only by a venous pressure increase the minimum calculated pressure (mean 14 2 range 12 1–21 5 cm H_2O) capable of causing these changes was higher than the above mentioned venous pressure increase *However the pressure changes on the arterial* side must be substantially larger (20–30 cm H O) than those on the venous side (10 cm H_2O) and this condition must contribute to the recorded volumetric changes on alteration of posture A slow type of volumetric change was seen even after 10 sec in the recumbent position The nature of this volumetric change as *well as that seen taking place even after a* 10 sec period of static pressure after a change in *middle ear pressure must await future re*search

Determinations of the volume of the middle ear as in procedure D are probably the first to have been made without any artefacts due to variation in volume of the mucosa (ΔV_{muc}) Table I clearly shows the risks of major errors when V_m is estimated without taking ΔV_{muc} into consideration especially when the middle ear volumes are small This is because C_{muc} is high in relation to V_m in such small middle ears perhaps because the mucosa of the middle ear is well vascularized compared with

ıt of the aır cell system The factor $\Delta P_m \times$ $_{uc}$ constitutes a large part of ΔV_m, eq 2 If ıs estımated according to procedure E, d C_{muc} ıs taken ınto consideration as ın eq ıt wıll gıve adequate ınformation about the ıe V_m If ın a gıven case C_{muc} ıs not known should be estımated which was earlıer done Ingelstedt et al (1967) The best estımatıon own ıs the equatıon $C_{muc}=0\ 45+0\ 061\times$ (Fıg 7) though ıt was based on only the esent 5 cases Takıng C_{muc} ınto considera- on gıves a closer estımatıon even when V_m ıs termıned ındırectly (Ingelstedt et al , 1967)

ZUSAMMENFASSUNG

schreıbung einer Methode zum Studıum der vom uck abhangıgen Veranderungen des Volumens der ttelohrschleımhaut Dıe Untersuchungen wurden beı terschıedlıchen Druckwerten ım Mıttelohr und ın der ıssenwelt durchgefuhrt Die druckabhangıgen Volu enanderungen der Schleımhaut waren beı Druck randerungen ım Mıttelohr durch Druckanderung dırekt der Pauke oder ındırekt ın der Aussenwelt dıe gleıchen ıt der beschnebenen Methode kann auch das Pauken lumen uber dıe Mıttelohrschleımhaut ohne Artefakte stımmt werden Das Volumen Druck Verhaltnıs ın der ıttelohrschleımhaut varıerte von 0 6–1 7 μl/cm H_2O und near mıt dem Volumen ım Mıttelohr Vergleıche vıschen Bestımmungen des Mıttelohrvolumens mıt und ıne Berucksıchtıgung der durch dıe Schleımhaut her orgerufenen Complıanz ergaben besonders beı kleınen auken deutlıche Unterschıede Der Eınfluss wechselnder Stellungen auf das Volumen der Schleımhaut wurde ebenfalls studıert Der physıologısche Mıtteldruck ıst ab- hangıg von der Funktıon der Eustachıschen Tube des Mıttelohrvolumens des Trommelfells und der Mıttel ohrschleımhaut Deshalb ıst dıe Bestımmung der Schleım hautcomplıanz wıchtıg für Berechnung des Mıttelohr drucks und auch für das Studıum des Volumens der luft gefüllten Mıttelohrraume Dıe Methode kann auch zur Klarung der Gefassfunktıon beı physıologıschen und krankhaften Zustanden sowıe der Reaktıon der Schleım hautgefässe auf Pharmaka beıtragen

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DENTINE AS AN OSSICULAR SUBSTITUTE

Experimental Results in Animals

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Abstract The fate of dentine grafts of autogenous,[1] allogeneic[2] and xenogeneic[3] origin has been studied in rats both in muscle and in the middle ear. The dentine has been implanted fresh, autoclaved, Cialit preserved and demineralised. Xenogeneic material tends to extrude from the ear and not to form a functional union with the structures it contacts. Fresh or Cialit preserved autogenous or allogeneic material eventually stimulate new bone formation and usually become incorporated into the host ossicular chain, however continuing resorptive activity is present. Autoclaved material does not result in new bone formation; demineralised allogeneic material stimulates very active new bone formation in the ossicular chain. It is concluded that non-demineralised dentine, on account of resorption, is inferior to ossicular grafts as a tympanoplastic material. Demineralised dentine, by virtue of its bone inductive potential, may be of value in tympanoplasty.

A previous report (Gibb & Stewart, 1972) described the background to our tentative introduction of dentine as an ossicular substitute in tympanoplasty. In summary, we found in dentine a material which was freely available, devoid of potential medicolegal problems and readily fashioned into convenient prostheses—in this latter aspect it promised to show a real advantage over the alternatives of allogeneic ossicles and cartilage. We had hoped that the biological origin of dentine would exempt it from the high extrusion risk of synthetic materials; we were also hopeful that antigenicity, resorption and the induction of excessive new bone would not give rise to problems.

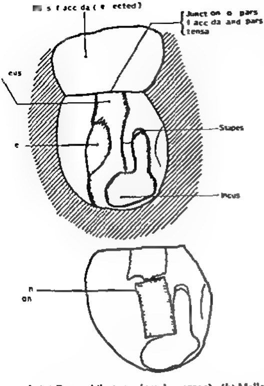
An unequivocal case of resorption of dentine in one of our early (and initially successful) clinical cases caused us to undertake animal experiments in order to obtain more basic knowledge of the outcome of dentine implants.

A series of detailed studies of incus grafts in rats have been carried out by van den Broek & Kuijpers (1967). They observed that immunological rejection phenomena with fresh incus allografts (as evidenced histologically by infiltration of the graft site by small lymphocytes) and also resorptive phenomena were prominent with grafts in muscle but did not occur in the middle ear. For this reason it was decided to implant dentine into both the middle ear and muscle. In the ear, experiments were designed to study the reaction to dentine when placed (i) against the tympanic membrane (in view of our previous experience of dentine resorption in this situation), (ii) against intact bone of both auditory ossicles and the promontory, and (iii) against recently fractured ossicular bone. Observations were also made on variations in preparation and preservation techniques.

[1] Autogenous: Derived from the same individual.

[2] Allogeneic: Derived from an individual of the same species but of a genetically different strain.

[3] Xenogeneic: Derived from an individual of a different species.



g 1 (*a*) Rat m ddle ear—dorsal approach (*b*) Malleus/ us graft in pos t on replac ng malleus head

At a later date stimulated by the experi s of Urist and his colleagues (1967 and 71) we carried out a series of short term ex eriments using demineralised dentine as a material

It may always be objected that experimental ings in rodents do not necessarily reflect the situation in man While we accept this we would point out that there is good correlation between experimental findings in rats and the clinical situation (van den Broek & Kuijpers 1967 Kerr & Smyth 1971)

MATERIALS AND METHODS

The recipients were young adult rats of the Wistar strain weight approximately 200 grams They were anaesthetised with barbiturate The implants were from three sources

(*a*) *Autogenous* (derived from the same individual) An upper incisor was fractured at its junction with the alveolar margin and removed The tooth was then broken up and fragments 0 5–1 mm in diameter selected Following examination with the microscope any enamel bearing fragments were rejected or modified A dental burr was used for removal of pulp and fine shaping

(*b*) *Allogeneic* (derived from an individual of the same species but of a genetically different strain) Obtained in a manner similar to (*a*)

(*c*) *Xenogeneic* (derived from an individual of a different species) Shaped from the healthy roots of human teeth any cementum or pulp substance being removed prior to implantation

Preparation variations

Drilling was carried out under saline (to prevent overheating) in all implants except those for autoclaving which were drilled dry

Preservation variations

(*a*) *Fresh implants* were stored in saline at 0°C and were used within 60 minutes of removal Certain series of fresh grafts were placed in penicillin solution for 20 minutes

(*b*) *Cialit preserved implants* were kept in Cialit 1 5000 aqueous solution at 4°C for 4–7 days

(*c*) *Autoclaved implants* were autoclaved at 135°C for 35 minutes

(*d*) *Demineralised implants* were obtained by placing small fragments of allogeneic dentine in 0 2 N hydrochloric acid at 4°C for 2 days

All grafts were washed in saline prior to use

Operative technique

The middle ear of the rat differs from that of man in that apart from the malleus handle, the entire ossicular chain lies within the attic region (Fig 1*a*) The stapes footplate is inaccessible surgically in the rat due to the fact that the stapedial artery is a major vessel which fills the space between the stapes crura The incus and malleus head are however readily accessible via a dorsal route—the pars flaccida may be displaced laterall

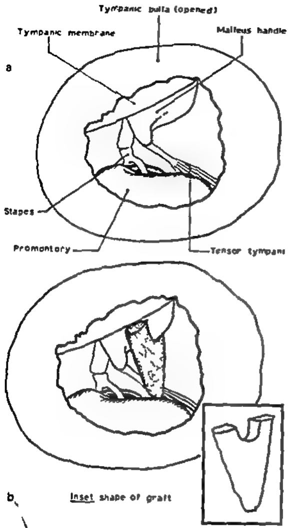


Fig 2 (a) Rat middle ear—ventral approach (b) Y graft in position

permitting access to the attic region An ossicular chain defect was created by removing the malleus head, a small dentine fragment was then used to bridge the gap between the recently fractured malleus neck and the incus (Fig 1*b*) There was a tendency for implants to contact the pars flaccida, as following surgical displacement this structure tended to take up a more medial position and lie loosely around the graft

As stated in the introduction it was considered desirable that grafts be studied when placed against the pars tensa or malleus handle The pars tensa is not accessible from the dorsal approach in the rat so it was necessary to operate via a ventral route by retracting the great vessels and removing muscle attachments from the tympanic bulla This could be opened by drilling It was then possible to place a short 'Y'-shaped implant between the malleus handle and the promontory (Fig 2*a*, *b*) This manoeuvre was unfortunately complicated in some cases by the fact that the malleus handle of the rat is keel shaped, rendering placement of grafts difficult In these cases the problem was overcome by placing a graft between the pars tensa and promontory employing a two-stage procedure At the first operation the malleus was removed per meatus by avulsing it with forceps In all

Table I *Experimental plan—number of implants*

Site and material	Preservation				
	Fresh	Fresh+ Penicillin	Autoclaved	Cialit	Demineralised
Muscle					
Autogenous	14	4	–	–	–
Allogeneic	12	5	11	12	5
Xenogeneic	15		12	12	–
Ear Malleus/Incus					
Autogenous	15	6	–	–	
Allogeneic	13		15	–	8
Xenogeneic	–	–	–	14	–
T piece Xenogeneic					
1 stage	–	–	–	14	–
2 stage	–	–		8	–

Usual survival times = 3 days 3 weeks 3 months 6 months 1 year 2 years

g 3 Fresh autograft muscle 8 weeks showing area of n with giant cell and maturing fibrous envelope aema oxyl n-eos n orig nal magn f cat on ×40

ases the tympanic membrane regenerated hin three weeks A T shaped graft could en be placed as described above Only hu an dentine was used in the manufacture of grafts as rat dentine proved unsuitable on unt of the small quantity available and its ability to fragment

Grafts were implanted in muscle by incising ver tibialis anterior and placing the graft di ectly into that muscle

The detailed experimental plan is depicted n Table I The rats were killed at intervals up o ? years The dentine bearing parts were dis out fixed in neutral formalin decalci ed in EDTA (10% at pH 7 4) mounted in araffin serially sectioned and stained Serial ections were cut of the whole of the dentine ants these sections being mounted in ro ation on three sets of slides which were ta ned by (a) haematoxylin-eosin (b) methyl green pyronine and (c) Heidenhain s azan The average specimen provided between one and two hundred sections

RESULTS

Non Demineralised Specimens

Implants in muscle

The initial reaction was that seen in any recent wound—red cells polymorphs and fibrin surrounded the implant A round cell response with lymphocytes and macrophages soon supervened Fibroblastic differentiation followed so that within 10 days the dentine was surr unded by an envelope of young fibrous tissue In c n m f the fresh gr fts which had not been treated with penicillin the polymorph response persisted longer presumably due to infection With the passage of time the fibrous

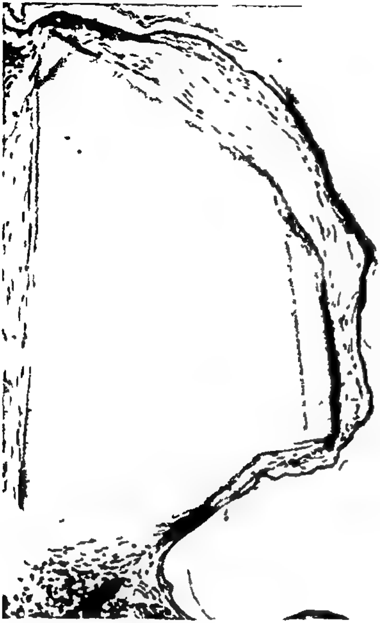


Fig 4 Calt preserved xenograft ? years Thickly encapsulated tenting pars flaccida Haematoxylin-eosin original magnification ×40

capsule matured and became quite thin with all types of graft (Fig 3)

Giant cells were visible in relation to all muscle grafts by 10 days Scanty at first they later became more prominent Although giant cells were usually in evidence at any site of major resorptive activity (Fig 3) resorption was also observed in places where highly pyroninophilic connective tissue cells were applied to the surface of the graft Resorptive activity appeared to be maximal in relation to irregularities in the dentine and at the sectioned ends of dentine tubules This may explain increase of resorptive activity around the autoclaved specimens (which tended to fissure and fragment as a result of this preparation technique)

Resorption was progressive and tended to increase with the passage of time By one to two years a difference was apparent between dentine of rat and human origin—the rat dentine grafts of all types were small and had un

Fig 3 Extrusion of graft through pars flaccida at 2 years Haematoxylin-eosin original magnification ×40

dergone considerable resorption—in some cases no grafts were found at the longer survival times The human dentine, by way of contrast, retained its gross integrity to a greater degree, microscopically, however, it was apparent that resorption was continuing It is problematical whether this difference between the two types of graft may be attributed to differences in dentine structure—rapidly growing rat incisor dentine is softer than human dentine—or to immunological differences

Although lymphocytes were visible in relation to all types of graft, particularly at the short survival times, these never became prominent and there were no differences between dentine of differing origins

No bone formation was seen in connection with any of the non-demineralised dentine implants in muscle

Response of the ear

The early red cell/polymorph response was in uninfected cases less well developed than in

Fig 6 Autoclaved allograft in ear one year Irregularly staining dentine marked resorption Haematoxylin-eosin original magnification ×40

uscle The dentine was rapidly covered by a fibro-epithelial envelope where it lay free middle ear cavity—it is remarkable that cells which later form this envelope were seen migrating over the graft as early as 3 days following implantation

Where dentine contacted tympanic membrane or ossicular bone its fibrous covering bound it to that structure

Resorptive activity was slow in the middle ear—*slower than with the muscle implants* Giant cells were present to a varying degree but were detectable to some extent in most cases Small erosions were seen at the earlier survival times these becoming more marked after one or two years Resorption was maximal in those areas of dentine which contacted other structures such as tympanic membrane or bone (as opposed to where dentine lay free in the middle ear air space)

Differences between the various types of dentine declared themselves as time progressed Cialit-preserved xenogeneic material became encapsulated by thick layers of mature fibrous tissue (Fig 4) Where it contacted ossicular bone or the promontory, this fibrous capsule separated dentine from the bone Resorption was progressive and there was a strong impression that all these grafts would extrude in time—in some ears extrusion was apparent (Fig 5) in others the dentine was seen tenting the tympanic membrane, in cases where there was no mechanical reason for this to happen (Fig 4) The overall picture was comparable with that seen with certain artificial implants—in one case where silastic sheeting was used to close the defect in the tympanic bulla, the host reaction to the Silastic was the same as that to human dentine

In one 10-day specimen of Cialit preserved

Fig 7 Decalcified allograft muscle 5 weeks Dentine replaced by area of new bone Haematoxylin-eosin original magnification ×40

human dentine, new bone was seen growing out from the recently fractured malleus neck to join the dentine No other case of new bone formation in relation to xenogeneic dentine was seen

The reaction to allogeneic dentine varied with the preservation technique The extreme degree of fibrous encapsulation seen with xenogeneic dentine did not occur Autoclaved material was progressively resorbed (Fig 6) and had virtually disappeared in some of the long survivals—there is no doubt about this although the histological evidence of active resorption was not dramatic in most instances

Where autoclaved allogeneic dentine contacted bone it was comparable to xenogeneic dentine, in that it tended to be separated from the bone by fibrous tissue

There was very little difference between fresh autogenous and allogeneic dentine as far as the reaction in the middle ear was concerned Moderate resorptive activity was seen in all cases Where these materials contacted bone the response differed considerably from that to autoclaved allogeneic or to xenogeneic material There was a definite tendency for new bone to form particularly where these grafts contacted recently fractured bone—this tendency increased with time In some instances the new bone grew into and replaced the dentine In others it would encapsulate the dentine In no case was the ossicular bone separated from the dentine by fibrous tissue

In a single case a portion of the root canal had been inadvertently included in a fresh allograft, new bone was seen in the roo despite the fact that it did n bone

Fig 8 Decalcified allograft malleus incus 5 weeks Only small fragment remains of dentine the rest having been replaced by bone and fibrocartilage which links malleus to incus Heidenhain's azan original magnification ×16

Demineralised Dentine

...ineralised allogeneic grafts have been ... only up to 7 weeks

Muscle grafts

Implants in muscle evoked a somewhat less vigorous inflammatory response than did any of the non-demineralised grafts The dentine tubules were rapidly invaded by extensions from host fibroblasts Demolition of the grafts appeared to be much more rapid than with non-demineralised material although giant cells were less in evidence There was induction of small amounts of bone in three out of five muscle implants of demineralised dentine studied to date (Fig 7) No new bone formation was seen in the control series where non demineralised implants from the same donors as the demineralised implants were placed in the opposite leg of the host

Middle ear grafts

Demineralised implants in the middle ear evoked a minimal inflammatory response In all eight grafts studied to date, new bone formation was seen in relation to the grafts The new bone formation was much more vigorous in general than was that seen with non-demineralised grafts It occurred in relation to the fractured malleus and the intact incus In two cases new bone was seen where dentine contacted the tympanic membrane In some instances there was proliferation of fibrocartilage more than bone, so that the grafts tended to become embedded in this tissue. In other cases the new bone grew into and replaced the dentine (Fig 8) A further pattern was the growth of new bone around the outside of the graft

In all cases where demineralised dentine

Table II *Summarising reaction to different graft types in ear*

Absent + Slight + Present ++ Marked

	Encapsulation	New bone	Extrusion
Xenogeneic	++	−	+
Allogeneic			
Autoclaved	±	−	
Fresh	±	+	−
Demineralised		++	−
Autogenous			
Fresh	±	+	−

was implanted functional union of the malleus remnant to the incus was seen

DISCUSSION

Progressive resorption of dentine occurred with all grafts being greater with autoclaved specimens presumably due to denaturation of the dentine resorption was less pronounced with human specimens possibly due to the fact that human dentine is structurally different and tends to become encapsulated by acellular fibrous tissue This finding of resorption contrasts with the experience of Zini in his clinical use of autoclaved allogeneic dentine grafts in tympanoplasty he states that he has seen neither macroscopic nor microscopic evidence of resorption in his human cases (Zini 1972) We ourselves have experienced resorption clinically and the evidence from this study is unequivocal however resorption of dentine in the middle ear is a slow process the histological detection of which may be difficult if serial sectioning techniques are not utilised In the respect of resorption dentine grafts appear to be inferior to ossicular grafts

As far as tissue tolerance of dentine is concerned the only real problem appears to be ultimate fibrous encapsulation and/or extrusion of xenografts from the middle ear Other types of graft provoke very little tissue reaction in particular the inflammatory response to demineralised allografts in the middle ear is minimal The classical histological evidence of an antigenic response namely lymphocytic infiltration round the grafts has not been observed to any significant extent either in the middle ear or in muscle with any type of graft This contrasts with the situation with ossicular implants in muscle (van den Broek & Kuijpers 1967) It may be that dentine has low antigenicity possibly due to the fact that it is acellular and that the protein of non demineralised dentine is relatively inaccessible to host cells However second set rejection of skin grafts has been demonstrated following heterotopic implantation of (*a*) whole tooth allografts (Schulman 1964 Valente et al 1964) and (*b*) demineralised dentine thus (Bang 1972) dentine obviously has some antigenic properties The lack of immune response to our grafts could be related to their small size and the fact that we were very careful to remove any pulp substance prior to implantation

New bone formation was seen in the middle ear particularly with demineralised dentine allografts and to a lesser extent with non-demineralised grafts of rat dentine which had not been denatured New bone was seen in muscle only with demineralised grafts

These findings require correlation with the results of other workers Urist and colleagues (1967 and 1971) have described a bone induction principle a constituent of the matrix of bone and dentine (probably an acid stable protein) which is capable of inducing uncommitted connective tissue cells to differentiate into osteoblasts or chondroblasts Demineralised dentine has been shown to be a particularly potent inducer of new bone This bone induction principle may be inactivated by heat infection and alkalis but is unaffected by acid or alcohol treatment or by freeze drying With incus allografts new bone formation is seen with fresh grafts and following preservation by alcohol cialit and fre drying boiling and formaldehyde prese destroy this osteogenic potential Broek & Kuijpers 1967 and 1974)

Our findings are in general compatible with this body of work Although our studies of demineralised dentine allografts in muscle have demonstrated clearly that the type of graft we used is capable of inducing new bone it is perhaps arguable whether the bone formation we observed in the middle ear was truly induced by the dentine—in many instances the grafts contacted recently fractured living bone The failure of bone formation with denatured grafts appears to suggest however that at least an element of bone induction was present Additionally it has been demonstrated (Morris 1971) that implantation of demineralised dentine together with fresh autogenous bone enhances bone induction by the dentine A further point in favour of the operation of the inductive mechanism in the present series is that bone was seen in two of the experiments where demineralised dentine contacted the tympanic membrane

Bone was found in relation to only one dentine xenograft Bone induction by both bone and dentine grafts has been shown to vary inversely with antigenicity being absent or scanty with xenografts (Bang 1972 Morris 1971 Narang et al 1971) It is apparent from these and from our studies that xenografts are generally effective in bone induction

demineralised dentine implants in muscle evoked only scanty new bone formation in a smaller proportion of cases than other workers have achieved (Yeomans & Urist 1967) We demonstrated no new bone whatsoever with non demineralised grafts where others have demonstrated scanty new bone formation in a proportion of cases (Bang & Urist 1967)

Two possible explanations must be considered

(1) We were meticulous in excluding pulp substance examining the grafts with the operating microscope and using the dental drill to remove any residual pulp substance (only a small proportion of the implants required drilling so heat denaturation is not the explanation —in any case precautions were taken to avoid this) Other workers appear to have been less careful in ensuring that the implant consisted only of dentine It is interesting that bone was present in the pulp-cavity of the single non demineralised graft which included pulp substance It has been demonstrated that osteoid forms to a greater degree in the pulp cavity of whole tooth implants (Irving & Band 1968 Paik et al 1972)

(2) The grafts were smaller than have been used by other workers

The ideal middle ear graft is either viable or ultimately replaced by viable host bone so that it becomes a functional part of the recipient where new bone forms it should not be so excessive as to cause ossicular fixation *This study lends support to others which have* helped to define the circumstances in which viable bone is induced in middle ear grafts (van den Broek & Kuypers 1974 Kastenbauer 1972)

In conclusion we consider that non-demineralised dentine will prove inferior in clinical use to currently used alternatives and especially to allogeneic auditory ossicles in the reconstruction of the ossicular chain We would re-emphasise however that resorption of dentine is a slow but continuing process so that permanent success can not be assumed until many years have elapsed following implantation

Demineralised dentine on account of its bone inductive properties may prove of value in the repair of small bony defects e g of the annulus posterior canal wall or possibly the ossicular chain It has been demonstrated (Register et al 1972) that demineralised dentine has the same bone inductive power in man as it has in experimental animals

ZUSAMMENFASSUNG

Das Verhalten autogener allogener und xenogener Dentin Transplantate nach orthotopischer und heterotopischer Transplantation wurde mit Tierexperimenten untersucht Frisches C alit kon erv ertes mit dem Autoklav steril ertes oder entkalktes Dentin wurde implantiert Zusammenfassend konnte f lgendes fe tge

stellt werden. Gegen xenogenes Dentin wurde ein Abwehrreaktion beobachtet. Frisches und Cialit konserviertes autogenes oder allogenes Dentin wurde in die Schalleitungskette inkorporiert und zeigte gegebenenfalls eine osteoinduktive Wirkung. Daneben wurde eine Resorption des Transplantats beobachtet, die kontinuierlich fortgesetzt wurde. Das im Autoklav sterilisierte Dentin zeigte keine osteogenetische Potenz. Entkalktes allogenes Dentin dagegen zeigte eine sehr starke osteoinduktive Leistung. Aus diesen Beobachtungen können wir schliessen, dass nicht entkalktes Dentin im Hinblick auf die Resorption den Gehörknöchelchen als tympanoplastisches Material unterlegen ist. Dagegen kann entkalktes Dentin durch seine osteogenetische Leistungsfähigkeit für den Aufbau der Schalleitungskette wertvoll sein.

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ACUTE FACIAL PALSY

Some Clinical and Virological Observations

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Abstract A prospective clinical and virological study on 44 patients with acute peripheral facial paralysis was carried out in consecutive cases during one year In 9 cases varicella zoster infections were serologically established In 5 additional patients an associated varicella zoster or herpes simplex infection was possible Of the 9 confirmed cases 6 were clinically diagnosed as zoster oticus whereas on clinical grounds 3 were regarded as Bell s palsy No evidence was obtained of associated entero virus mumps measles cytomegalovirus tick borne encephalitis virus para influenza virus mononucleosis or *Mycoplasma pneumoniae* infection

peripheral facial paralysis may occur in ation with vesicles and vestibular oms—herpes zoster oticus In some of these cases the zoster infection has been serologically confirmed (Aitken & Brain 1933 Petersen & Caunt 1970 Tomita et al 1972) However acute facial paralysis without any other symptoms is far more common The etiology in such cases—in the literature often called Bell s palsy—is unknown Ischemia (Miehlke, 1973) or a retrograde spread of inflammation along the chorda tympani to the facial nerve has been postulated (Sade 1965 Blatt & Freeman, 1966) Viral infections have also been associated with acute idiopathic facial paralysis Herpes zoster infections may occur without clinical signs other than facial paralysis, as suggested by Tomita et al (1972) The clinical data in their study were not quite clear on this point Other viral infections (e g mumps, mono nucleosis) have in scattered cases been associated with facial palsy (Bernstein & Wolff, 1950, Saunders & Lippy, 1959, Taylor & Parsons-Smith, 1969, Liebscher & Wunderlich, 1966, Mendonca, 1971) whereas previous prospective investigation concerning respiratory virus, hepers simplex and mumps infections in facial palsy were negative (Korczyn et al , 1973, Brackman, 1974)

Against this background the present prospective study was started The purpose of the study was to evaluate a possible association between acute, peripheral facial paralysis and virus infections that could be diagnosed by a broad virological examination, which included investigations for varicella zoster infections The study lasted a whole year so as to cover any seasonal variations

MATERIALS AND METHODS

Patients at the ENT Department, Sabbatsbergs Hospital in Stockholm having peripheral facial paralysis during the period November 1970 through October 1971 were subjected to a clinical and virological examination Pa

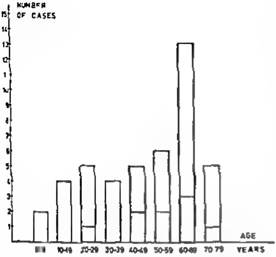

Fig 1 Distribution of 44 cases of facial paralysis according [redacted] age Shadowed areas indicate serologically confirmed cases of varicella zoster infection

tients with intracranial tumours systemic neurologic disorders, traumatic paralysis, otitis and for practical reasons newly delivered mothers were not included in the study

The case material, comprising 44 patients (19 males and 25 females) may on clinical grounds be divided into three groups

(*a*) patients with peripheral facial palsy combined with vesicles on the external ear or in the external auricular canal and/or symptoms of vestibular disorder in this paper termed clinical Herpes Zoster oticus (7 patients)

(*b*) patients with facial palsy without vestibular or external ear symptoms but with a history of blisters in the mouth preceding the occurrence of facial palsy (4 patients)

(*c*) patients with facial palsy without any history, or previous clinical symptoms or signs of illness other than the paralysis (33 patients)

Clinical examination

The patients were subjected to [redacted] routine ear nose and throat examination and routine blood tests In patients with vertigo or suspected vertiginous disease, vestibular examination including caloric stimulation with electronystagmographic registration was performed In 32 out of the 44 patients [redacted] lumbar puncture was made in order to examine cells and protein contents, and for the virus study of the cerebrospinal fluid (CSF) (12 patients refused lumbar puncture) A cell count exceeding 5 white blood cells per mm³ was regarded as pleocytosis

Virological examination

Specimens for virus isolation and serological examination were taken at the first visit (in 38 cases within one week after onset of illness, in the remaining 6 cases between 7 and 13 days) Later blood specimens were drawn 2 4 and 8 weeks after the onset of illness

Virus isolation

Fecal specimens from 24 patients and throat specimens from 17 patients were tested in tissue culture (GMK AH I HeLa and primary cynomolgous monkey kidney cells Fransen et al 1969) In order to test for coxsackie virus group A newborn mice were inoculated with fecal specimens from 7 patients taken [redacted] in August or September [1] Cerebrospinal fluid from 30 patients was tested in tissue culture (GMK AH I primary monkey kidney cells Wolontis & Bjorvatn 1973)

Serological examination

Serological examinations were performed by complement fixation (CF) tests (Fulton & Dumbell 1969 Svedmyr et al 1957) A significant increase [redacted] antibody titres (≥4 fold) obtained within one month after onset of illness

mumps measles herpes simplex (Fransen et al 1969) varicella zoster (v z) (Svedmyr 1966) cytomegalovirus tick borne encephalitis (TBE) (Svedmyr et al 1958) and a commercial mycoplasma pneumoniae antigen (Orion Oy Finland) Mononucleosis tests were performed in sera from 37 patients screen tests Monospot if positive (2 patients) followed by Paul–Bunnel–Davidsohn (Davidsohn 1937 Lee et al 1968 Wahren 1969)

RESULTS

Clinical findings

The study comprises 44 patients (19 25 females) The age distributio ranging from 7 to 77 years

[1] We are indebted [redacted] Dr Annelise Godtfredsen and Maria Pedersen Statens Seruminstitut Copenhagen for doing the mice inoculations

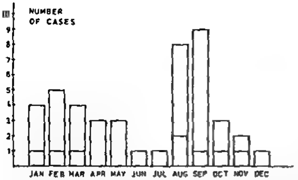

Fig 2 Distribution of 44 cases of facial paralysis according to month of onset of illness Shadowed areas indicate serologically confirmed cases of varicella zoster infection

small material a slight prevalence was noted in group 60–69 years The seasonal distribution is shown in Fig 2

The clinical data are summarized in Table I Seven patients had—in addition to facial palsy—changes on the external ear (5 patients) or vestibular disorders (in 3 patients) One of the patients also had a history of blisters in the mouth preceding the occurrence of the paralysis Examinations of the cerebrospinal fluid from 6 of these patients revealed pleocytosis in 5 with a total cell count range of 15 to 252 cells/mm³ predominantly monocytes The protein content was normal The clinical diagnosis for these 7 patients was herpes zoster oticus

Four patients had no clinical signs of external ear changes or vestibular disorder, but had a history of vesicles in the mouth preceding the onset of paralysis The cell count and protein content were normal in the cerebrospinal fluids available from 2 of the patients

The remaining 33 patients showed facial paralysis without any history clinical signs or symptoms of preceding or concomitant infection and presented no symptoms of vestibular disorder Cerebrospinal fluid was available from 24 patients pleocytosis was demonstrable in 4 whereas the findings for the remaining 20 patients were normal

Virological results

No virus was isolated from any material tested (stools, throat specimens, cerebrospinal fluid)

The serological examinations, by CF tests against enterovirus, mumps, measles, para influenza, TBE, cytomegalovirus and *Myco plasma pneumoniae* antigens were unrewarding In about half of the patients either very low, or no, antibody activity to enterovirus, measles, para influenza I and 2 and *Mycoplasma pneumoniae* antigen was detectable The same was valid for mumps in about two thirds of the patients Antibody activity to tickborne encephalitis virus was not demonstrable in any sera, and the mononucleosis tests were negative

Significant titre rises were, however, demonstrable against v-z antigen in 9 patients In 2 of these a concomitant titre rise against herpes simplex antigen was noted neutralizing antibody activity to this virus remained stationary, making a v-z infection more probable In 5 additional patients stationary CF antibody

Table I *Relationship between clinical picture and titres against Varicella-Zoster antigen in 44 cases of peripheral facial paralysis (figures given indicate numbers of cases)*

	Facial paralysis combined with		
	Herpes zoster oticus	History of vesicles	No other symptoms
Total number of patients	7	4	33
External ear changes	5	0	0
Vestibular symptoms	3	0	0
No of CSF examined	6	2	24
Pleocytosis (15–252 cells/mm³)	5	0	4
Complement fixing antibody activity to Varicellae zoster			
Significant increase	6	0	3
Constant level ≥64	0	3	2
Constant level 8–32	1	0	7
Level <8	0	1	21

activity against v-z antigen was found at a high level (≥64) Eight of the 9 patients with v-z infection were more than 40 years of age (see Fig 1) and the onset of illness occurred at all times of the year (see Fig 2)

The correlation between v-z serology and clinical data is given in Table I In 6 of the 7 patients with a clinical diagnosis of herpes zoster oticus the serologic results confirmed the diagnosis The seventh patient had constant antibody activity to v z at a fairly high level (titre 32)

Cerebrospinal fluid for virus isolation was obtained from 5 of these patients Attempts to isolate v-z virus was unrewarding, although 4 of the patients had pleocytosis as a sign of meningeal involvement

Of the 4 patients with a history of blisters in the mouth preceding the onset of palsy but no external ear changes or vestibular disorder, 3 showed high stationary antibody activity (titre ≥64) to both v-z and herpes simplex antigen The fourth patient, a girl aged 11 years, had herpes simplex antibodies but no demonstrable antibody activity to v z She had a history of recent vesicles in her mouth, and similar cases had occurred in her surrounding

Among the 33 patients with facial palsy as the only symptom significant antibody increase against v-z antigen was demonstrable in 3 patients In 2 additional patients a high activity to v-z and herpes simplex was demonstrable Twenty one patients had only low or no antibody activity to v z There was no correlation between pleocytosis and v z infection in the 4 patients with pleocytosis no or very low anti varicellae activity was present whereas in the two available CSF samples in serologically confirmed zoster infections cell count and protein content were normal

DISCUSSION

Of the 44 patients suffering from peripheral facial paralysis in this material 7 had clinical zoster oticus As in other studies (Tomita et al 1972) the serological investigation confirmed the zoster diagnosis (in 6 of the 7 patients, whereas in the seventh patient it neither contradicted nor established a v z diagnosis) Most of these patients had pleocytosis in their cerebrospinal fluid We were not successful, however, in isolating the v-z virus Such isolations seem to be rare, but have been reported (Gold & Robbins, 1958)

In the patients who had a history of preceding blisters but no ear changes, the time interval between the onset of infection and the facial paralysis diminished our chance of obtaining a confirmatory serologic diagnosis The high antibody levels against v z or herpes simplex antigen do not contradict infections with either virus The antigenic relationship between the two antigens (Kapsenberg 1964, Svedmyr, 1965) makes serological differentiation difficult, and material for virus isolation was not obtained from blisters

About three-quarters of the patients had facial palsy as single symptom—Bell's palsy In these patients too, serological evidence of zoster infection was obtained in a certain number It may be noted that these patients lacked typical signs of zoster oticus and neither cell count in CSF nor in blood indicated infection These observations confirm earlier suggestions on this point (Tomita et al , 1972)

In the major part of patients with Bell s palsy no indication of associated v z infection was obtained Rather did the negative, or low antibody activity, seen in 21 of the patients make associated, generalized infection with this agent unlikely A localized infection in the facial nerve not accessible to antibody forming cells cannot be excluded, and cannot be diagnosed by the techniques used in the present investigation This applies also to recurrent herpes simplex infections The existence of localized infections has been suggested by the finding of an experimental study of herpes simplex virus in rabbits (Kumagami, 1972)

Judging from clinical experience over a period of several years, cases of facial p[illegible] to increase during early autumn This observation raises the qu[illegible]

viral or tick-borne encephalitis virus etiology However, no support for enteroviral infections was obtained by virus isolation or serological results Moreover, none of the patients showed any evidence of past infection with tick-borne encephalitis virus Also the investigation for mononucleosis, in some cases reported to be associated with facial palsy (Bernstein & Wolff, 1950, Mendonca, 1971), was negative in our study

No proof was obtained of associated infection with the other agents tested It was of interest to note that as many as 2/3 of the patients had either very low, or no antibody activity to mumps, thus contradicting recent infection In the older literature, mumps had been postulated as a common cause of Bell's palsy (Saunders & Lippy, 1959) but this observation has not been confirmed

Summarizing our data, we have found v-z infections to be associated with some cases of facial paralysis but no clue to any other possible viral etiology was obtained With the present technical approach this is in line with the lack of an established viral etiology in some cases of aseptic meningitis with or without accompanying cranial nerve paralysis (Skoldenberg 1972)

ZUSAMMENFASSUNG

[illegible] prospektive klinische und virologische Untersuchung wurde wahrend eines Jahres an 44 konsekutiven Patienten mit akuter peripherer Facialislahmung durchgefuhrt Bei neun Fallen wurde eine Zoster Infektion serologisch bestatigt Ein assoziierter Varicellae Zoster oder Herpes simplex war bei weiteren funf Fallen moglich Von den neun bestatigten Fallen wurden aus klinischen Grunden sechs als Zoster Oticus und drei als Bell s Paralyse Patienten betrachtet Eine Infektion mit Enterovirus Parotitis Masern Cytomegalo-Virus Fruhsommer Encephalitis Virus (TBE) Parainfluenza Virus Mononucleosis oder *Mycoplasma pneumoniae* konnte nicht bestatigt werden

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ELIMINATION OF CONTRAST MEDIUM FROM THE MAXILLARY SINUS

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Abstract. Drainage of contrast medium from the maxillary sinus during blowing and sniffing was studied by cine roentgenography in 11 healthy subjects. The functional size of the maxillary ostium was measured by a manometric method. Sniffing facilitated drainage more than blowing but the effect was minimal for both procedures except when sniffing was performed with the head tilted to the side opposite the filled sinus. The latter procedures may trap air in the sinus above the level of the ostium and thus facilitate drainage during sniffing. Another facilitating factor which is discussed is that blowing the nose may catch tenacious mucus which has partly passed through the ostium by the ciliary activity in the sinus. The influence of the fluid viscosity is discussed. The time required for complete emptying of the sinus from contrast medium, studied over a period of several days, was found to be inversely correlated to the functional diameter of the maxillary ostium.

The Bernoulli theorem has been discussed in connection with ventilation and drainage through the ostia of the paranasal sinuses (Colson, 1945; Proetz, 1953). Bernoulli's theorem, a principle in physics applicable to fluid dynamics, states that for a liquid flowing through a straight tube with a smooth constriction the internal fluid pressure is minimal at the constriction where the fluid velocity is greatest. The water suction pump is an application of this theorem.

When Bernoulli's effect is applied to the nose and paranasal sinuses it has been assumed that the region of the ostia is the narrowest part of the nose. Colson (1945) suggested that the passage of mucus from the sinus to the nasal cavity would be caused by this effect. Proetz (1953) denied such a possibility since there are no pronounced constrictions in this area and also due to the observation that the measured pressure changes in the maxillary sinus are equal to those in the nasal cavity.

The fact that the pressure changes are identical in the maxillary sinuses and the nasal cavity on breathing and forced respiration has been verified in several hundred maxillary sinuses examined in Uppsala. This observation is valid for air filled sinuses, but it seems likely that it also is valid in the presence of mucus. However, the Bernoulli effect cannot be definitely excluded when there is mucus in the sinus. In favour of such an hypothesis would be the clinical observation that patients with sinusitis sometimes can blow out large quantities of mucus even if the nasal cavity is initially free.

Takahashi et al. (1971) made an extensive roentgenographic study of the maxillary ostium after filling the maxillary sinus with contrast medium. The different filling defects in the sinus combined with the presence or absence of contrast medium in the natural orifice of the sinus were used for classification of various disorders. The time required for emptying the maxillary sinus after filling with contrast medium has been studied by several

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authors (King 1935 Larroude 1948 Kunzel 1952 Flottes et al 1960 Nucci 1964 Montserrat Viladiu et al 1967 Takahashi et al 1971) The reported times varied considerably partly due to the contrast medium (water or oil soluble) and its viscosity partly to pathological changes in the sinus (Flottes et al 1960)

Studies of the immediate effect of blowing or sniffing have not been performed and all investigations published so far have employed conventional roentgen techniques and not cine roentgenography

The aim of the present investigation was to study the presence or absence of the Bernoulli effect during sniffing and blowing by cine roentgenography after filling the maxillary sinus with contrast medium

The investigation was performed on subjects whose functional maxillary ostia sizes were measured according to a recently published method (Aust & Drettner 1974) Furthermore the time required for complete emptying of the sinus was studied and related to the size of the maxillary ostium

MATERIAL AND METHODS

Eleven subjects (8 men and 3 women) in the ages 20 to 32 years were investigated of whom 10 were healthy without nasal infection or allergy and normal rhinoscopy and roentgenography of the paranasal sinuses

The functional size of the maxillary ostium was first measured by introducing two puncture needles into the maxillary sinus one being used for the introduction of a brief air stream of 1 2 or 4 l/min and the other for pressure recording (Aust & Drettner 1974) The increase of pressure was transformed to functional size of the ostium by using a nomogram obtained from model experiments After this procedure one of the puncture needles was removed and the other was used for filling the maxillary sinus with a contrast medium Positive contrast medium in 1 2 3 or 4 portions of 5 ml each was used The contrast mediums were Endografin and Angiografin® in 8 subjects having viscosities of about 50 and 10 cps respectively at 20°C The sinus was filled to the level of the middle meatus

The roentgenographic examinations were performed in an erect position The focus film distance was 120 cm the object film distance about 30 cm and the focal spot 0 6×0 6 mm Cine roentgenography was performed in an anterior–posterior projection with 20 frames per second The tube potential was about 90 kV and the exposure time 0 003 sec A 9 cesium iodide image intensifier (Sirecon Siemens) was used in conjunction with a 35 mm camera (Arriflex Siemens) Films were taken during sniffing and blowing and examinations were performed after each 5 ml portion of contrast medium In two instances films were also taken with the subject s head tilted to one side

For the calculation of the volume of the maxillary sinus according to a previously published method (Aust & Helmius 1974) plain films at 80 kV in the anterior–posterior and lateral projections were taken with a ruler postured in the plane of the maxillary sinus

The time required for emptying of the sinus was investigated roentgenographically with plain films taken daily after the contrast administration until the contrast completely disappeared

RESULTS

Roentgenographic examination with contrast medium sometimes gave information which was not apparent from the ordinary plain films prior to filling with contrast medium Fig 1 shows a case with a ridge within the sinus and this anomaly was not visible on ordinary films

Drainage of contrast medium from the maxillary sinus during sniffing or blowing occurred rarely (Table I) and only in small quantities One subject had some drainage through the maxillary ostium both during blowing and sniffing It was remarkable that this su had the narrowest ostium of the whole

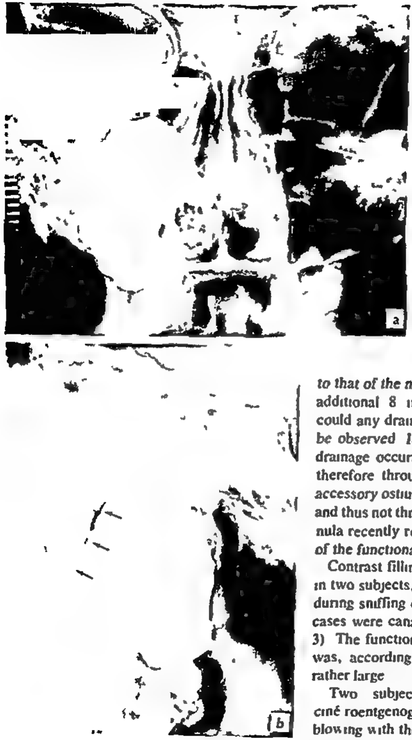

Fig 1 Maxillary sinus filled with Angiografin* Septum in the antero-superior part of the sinus (*arrows*) Straight antero-posterior (*a*) and lateral (*b*) projections

Two other subjects had slight drainage of contrast medium during sniffing but not during blowing and these had ostia of medium size (Fig 2) The drainage in these cases occurred only when the sinus was filled to a level close to that of the maxillary ostium In none of the additional 8 investigated maxillary sinuses could any drainage during sniffing or blowing be observed It is worth mentioning that the drainage occurred in the middle meatus and therefore through the natural ostium (or an accessory ostium), but not in the lower meatus and thus not through the hole made by the cannula recently removed after the measurement of the functional size of the maxillary ostium

Contrast filling of the ostium was obtained in two subjects, neither showing any drainage during sniffing or blowing The ostia in these cases were canals and not only orifices (Fig 3) The functional ostium size in these cases was, according to the manometric method rather large

Two subjects were investigated with ciné roentgenography during sniffing and blowing with the head tilted to the side opposite the filled sinus In these two cases there was drainage of contrast medium during sniffing (Fig 4), but not during ordinary breathing In one instance there was also slight drainage during blowing but less than during sniffing The ostial sizes in these two subjects were at each end of range in the series Two additional

Table I *Results of measurements of volume of the maxillary sinus, functional size of the maxillary ostium and of different parameters studied after contrast medium instillation in the maxillary sinus in 11 healthy subjects*

Subject	Maxillary sinus volume (ml)	Functional ostium diameter (mm) (erect position)	Contrast medium	Visualization of maxillary ostium	Drainage		Drainage with tilted head			Time for complete emptying (days)
					Sniffing	Blowing	Spontaneous	Sniffing	Blowing	
1	15 5	1 1	Angiografin	–	+	+	–	+	+	2
2	10 1	1 1	Endografin	–	–	–				Irrigated
3	7 8	1 8	Endografin	–	+					4
4	27 3	1 9	Angiografin	–	–					1
5	20 2	2 3	Angiografin	–	–	–	+			1
6	24 9	2 3	Angiografin	–	–					3
7	13 8	2 4	Angiografin	–						3
8	27 3	2 6	Endografin	+	–	–				2
9	24 9	2 8	Angiografin	+	–	–	–	+	–	1
10	26 2	3 4	Angiografin	–	+					1
11	23 5	5 6	Angiografin	–		–	+			0
Mean	20 2	2 5								

subjects noted spontaneously that contrast medium was pouring from the sinus when they tilted the head after the investigation One of these had the largest ostium in the whole series and the other an ostium of medium size

Since plain roentgenographic films were taken every day until the sinus was empty, the duration of this emptying of the sinus could be analysed in relation to the size of the maxillary ostium Fig 5 shows that the emptying process usually occurred more rapidly when the ostia were large The correlation coefficient in this relationship, which appeared to be linear, was 0 80, which is significant No difference in emptying time could be correlated to the two

Fig 2 Ciné roentgenographic frame of contrast filled maxillary sinus in straight antero-posterior projection Drainage of contrast medium (*arrows*) during sniffing

Fig 3 Ciné roentgenographic frame of contrast maxillary sinus and ostium (*arrows*) d Straight antero-posterior projection

Fig 4 Cine roentgenographic frame of contrast filled maxillary sinus in inclined anterio-posterior projection Drainage of contrast medium (*arrows*) during sniffing

different contrast mediums used in this investigation

DISCUSSION

Roentgenexamination of contrast filled maxillary sinuses has previously been made for studying the time required for emptying of the sinus and for investigations of the shape of the sinus as well as of mucosal swelling In the investigation performed by Takahashi et al (1) the latter was combined with roent- studies of the maxillary ostium The present investigation has also included such studies, although the main interest has been directed towards the drainage of the maxillary sinus and the possible importance of Bernoulli's effect

Visualization of the ostial canal without simultaneous drainage from the sinus was only found in 2 of the 11 subjects The ostia in these 2 subjects were not only orifices but real canals

The effect of sniffing or blowing on the drainage of contrast medium from the sinus was very small This was especially true for blowing, during which only one subject had a slight drainage During sniffing this subject and two others had drainage It seems thus that drainage may occur more easily during sniffing than blowing, but under both conditions only small amounts are excluded

A requisite for this drainage was that the sinus was filled to the level of the maxillary ostium No drainage was thus obtained when the sinus only contained a small amount of contrast medium However, the sinuses were never completely filled with contrast medium in order to avoid passive drainage

The viscosity of the fluid, within the limits of the two contrast media used in this investigation, appeared to have no influence on the drainage The viscosity of the contrast media was, however, considerably lower than that of mucus from the sinus, which according to Zippel et al (1974) is at least about 125 cps It seems probable that drainage through the ostium during blowing and sniffing will be facilitated by a lower fluid viscosity This investigation thus probably overestimate drainage during blowing and sniffing

It can thus be concluded that the Bernoulli effect has no practical importance for drainage from the maxillary sinus during ordinary blowing or sniffing However, drainage from

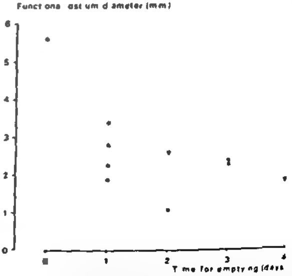

Fig 5 Functional diameter of the maxillary ostia in relation to the times required for complete emptying of the maxillary sinuses after filling with the contrast mediums Angiografin® (●) or Endografin® (▼)

the maxillary sinus may still be facilitated under special circumstances Firstly, it is possible to pour out fluid from the sinus by tilting the head to one side Such a drainage can be obtained especially when the ostium is large, but sometimes also with small ostia The greater viscosity of mucus, however, probably functions as a hindrance to drainage Thus, such emptying of the sinus only by tilting the head is probably of no practical importance

Another condition that can facilitate drainage is sniffing with the head tilted to the side opposite to the filled sinus In this position the fluid level is above the ostium and air is trapped in the sinus During sniffing the air in the sinus *will have a higher pressure than in the* nose and drainage may be obtained Blowing will probably not have a similar beneficial effect Such a condition with fluid in the maxillary sinus slightly below the level of the ostium is probably rather common during acute sinusitis It is required that the ostium is patent and that the mucosal swelling in the sinus is not so pronounced that air is excluded above the level of the fluid

Still another factor may be working during a sinusitis episode when viscous mucus may be pressed through the ostium by ciliary activity, like tenacious tooth paste coming out of a tube (Proetz, 1953) If the patient blows his nose during such a condition, the air coming through the nose may catch the mucus on the nasal side of the ostium and draw out additional mucus from the maxillary sinus Due to the low viscosity of the contrast media such a procedure could not be tested, but clinical observations support this theory

The time required for emptying of contrast medium from the maxillary sinus has previously been investigated by several authors In the present investigation this emptying time has been studied in subjects whose functional maxillary ostia size was measured An inverse correlation between the size of the maxillary ostium and the time required for emptying of the sinus was found

The relation between the amount of resorbed contrast medium and that which was eliminated through the ostium has not been analysed in the present series If the sinus could be assumed to be spherical, the volume and the surface area should have a relation of r/3, meaning that this relationship is larger in a sinus with great radius than in one with small If it also is assumed that the sinus is half filled with contrast medium and furthermore that the resorption per surface unit is constant, the resorption to a complete emptying without drainage through the ostium should require a longer time in a large sinus than in a small The result (Table I) is in favour of the opposite, i e emptying takes a long time in sinuses with a *small volume The resorption is thus of less* importance for the present results than the size of the maxillary ostium, which has a considerable influence on the drainage from the sinus not only instantly but also during a more protracted period

ZUSAMMENFASSUNG

Dranage von Röntgenkontrastmittel aus der Kieferhohle besonders beim Schneuzen und Schnuffeln wurde mit Hilfe von Rontgenkinematographie an 11 gesunden Personen studiert bei denen auch die Grosse des Kieferhohlenostiums mit einer manometrischen Methode festgestellt wurde Beim Schnuffeln erzielte man haufiger eine Dranage als beim Schneuzen wenngleich beide nur eine geringe Dranage ergaben Wenn man beim Schnuffeln jedoch den Kopf auf die der mit Kontrastmittel gefüllten Kieferhohle gegenüberliegende Seite neigte war die Dranage besser In dieser Stellung kann die Luft die sich in der Kieferhohle oberhalb des Ostiumniveaus befinden kann die Dranage beim Schnuffeln bewirken Ein weiterer Faktor ist der Umstand dass der Luftstrom beim Schneuzen zahen Schleim fangen kann der durch die Zilienaktivitat in der Kieferhöhle das Ostium zum Teil passiert hat Die Bedeutung der Viskositat der Flussigkeit wird auch diskutiert Es wurde die Zeit studiert die zur spontanen vollständigen Entleerung von Rontgenkontrastmittel aus der Kieferhöhle benotigt wurde Dabei wurde festgestellt dass diese Zeit umgekehrt proportional zum funktionellen Durchmesser des Kieferhöhlenostiums ist

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THE ANTIBACTERIAL EFFECT OF ANTIBIOTICS IN TREATMENT OF MAXILLARY SINUSITIS

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Abstract The antibacterial effect of antibiotic treatment in maxillary sinusitis has been assessed by studying the elimination of bacteria from the maxillary sinus during treatment with penicillin and tetracycline As adequate antibiotic concentrations are the prerequisite for maximal antibacterial effect the antibiotic concentrations were controlled The antibiotic concentrations were determined in maxillary sinus secretions and/or mucosas of 113 patients with maxillary sinusitis treated with single or repeated doses of penicillin or tetracycline or the two antibiotics in combination In 30 patients treated with either penicillin or tetracycline the bacterial growth in sinus secretion was controlled before and after treatment It was established that ordinary clinical doses of penicillin and tetracycline may although not invariably result in antibiotic concentrations in sinus secretion and sinus mucosa that can be regarded as adequate for treatment i e for penicillin ≥0 25 μg/ml and for tetracycline ≥1 0 μg/ml The necessity of achieving adequate antibiotic concentrations in sinus secretion and sinus mucosa to ensure therapeutic success was demonstrated Thus bacterial growth remained in the secretions of most maxillary sinuses when the concentrations of penicillin or tetracycline in the secretions were not regarded as adequate for treatment In contrast the bacteria were exterminated in most cases where concentrations were regarded as adequate

Bacterial studies have shown that in cases of maxillary sinusitis pathogenic bacteria are found in the secretions of 60–70% of the diseased sinuses (Kortekangas 1964, Nylen et al, 1972 Axelsson & Brorson 1972 Rantanen & Arvilommi 1973) Pneumococci and Haemophilus influenzae almost equally common taken together are present in about 50% of the case The remaining bacterial findings consist of bacterial species such as β streptococci staphylococci, coli-like organisms and anaerobic bacteria Pathogenic bacteria have not been found in healthy maxillary sinuses (Bjorkwall, 1950) These bacteriological observations show that a large proportion of maxillary sinusitis cases ■ caused or maintained by the invasion of the sinus by bacteria Thus, it can be expected that treatment with antibiotics to which the prevailing bacteria are sensitive, would improve the healing event in maxillary sinusitis

To establish the accuracy of this assumption a number of antibiotics have been tested in clinical trials which sought to assess antibiotic effect from the disappearance of one or more clinical symptoms of maxillary sinusitis (Kortekangas 1964 1965 Jeppesen & Illum, 1972, Jeppesen & Schaldemose 1970, Rantanen & Arvilommi 1973, Reynolds et al, 1964, Axelsson et al 1970 1971, 1973, Lumio, 1956)

The results of the clinical trials so far performed are not concordant It is difficult to ascertain the reasons for this divergence in results However in these clinical studies the antibiotic concentrations were controlled neither in serum in sinus secretion nor in sinus mucosa during treatment Therefore it is not to be relied on that the prescribed tic treatment resulted in adequate a concentrations at the site of the Thus inadequate antibiotic cc

be one cause of therapeutic failure Another source of erroneous results can be that the parameter used was not suited to its purpose The anticipated and specific effect of antibiotic treatment of maxillary sinusitis is the elimination of the bacteria This does not invariably mean the elimination of the clinical signs of the sinusitis These signs may have other causes than bacterial infections of the mucosa and therefore cannot be used in evaluating the effect of the antibiotic used

To assess the effect of antibiotics in the treatment of maxillary sinusitis more accurately than has hitherto been the case the concentrations of penicillin and tetracycline during treatment were determined in serum sinus secretion and sinus mucosa The antibiotics were administered either one by one or in combination as a single dose or as repeated doses The sinus secretions of patients treated either with penicillin or tetracycline alone were examined for bacterial growth both prior to and during the course of the antibiotic treatment

MATERIAL

Patients

Samples of maxillary sinus secretion and/or maxillary sinus mucosa from 113 patients treated with penicillin and/or tetracycline for maxillary sinusitis have been investigated

Seventeen patients representing 20 diseased maxillary sinuses were studied with regard to the concentrations of penicillin and tetracycline in maxillary sinus secretion after a single dose (Lundberg & Malmborg 1974)

Forty nine patients representing 54 diseased maxillary sinuses were studied with regard to the concentrations of penicillin and tetracycline in maxillary sinus secretion after repeated doses (Lundberg & Malmborg 1973) In 17 of the patients treated with penicillin and in 13 of the patients treated with tetracycline the bacterial growth in the secretions was studied both before and after treatment

Forty seven patients were studied with regard to the concentrations of penicillin and tetracycline in maxillary sinus mucosa after a single dose In 15 patients the concentration of penicillin and tetracycline could also be determined in corresponding sinus secretions (Lundberg et al 1974)

Antibiotics

The patients received either penicillin or tetracycline or the two drugs in combination Penicillin V (Meropenin Kabi) and tetracycline HCL (Tetracyn Pfizer) were administered orally Benzylpenicillin (Bensylpenicillin Kabi) procaine benzylpenicillin (Suspenin Kabi) and tetracycline hexametaphosphate (Tetradecin Novum Astra) were given intramuscularly

METHODS

Sampling procedures

Maxillary sinus secretion Sinus secretion samples were obtained by aspiration through a Lichwitz needle The secretions were classified as purulent or mucous according to their macroscopic appearance (Lundberg & Malmborg 1973)

Maxillary sinus mucosa Samples of maxillary sinus mucosa were obtained from radical antrum surgical intervention (Caldwell Luc) or in connection with transantrosphenoidal hypophysectomies where the sinus mucosa in any case is sacrificed

Serum A venous blood sample was taken from the cubital vein of the patient and allowed to clot before the serum was drawn off

Bacteriological analysis The antibiotic concentrations in maxillary sinus secretion maxillary sinus mucosa and serum were determined by an agar diffusion method with paper discs as diffusion centres (Lundberg & Malmborg 1973 Lundberg et al 1974)

The samples of aspirated sinus secretions were examined immediately for bacterial growth by the conventional agar plate technique in routine use at the Microbiological Department of Karolinska Sjukhuset

Definition of adequate antibiotic concentrations in maxillary sinus secretion and mucosa

This definition is based on an international collaborative study of antibiotic sensitivity testings where it is recommended to categorize bacterial sensitivity into four groups 1–4 in decreasing bacterial sensitivity (Ericsson & Sherris 1971) Antibiotic concentrations equal to or above the upper limit of MIC (Minimal inhibitory concentration) for bacteria categorized as sensitive to the antibiotic used group 1 are defined as *adequate concentrations* At Karolinska Sjukhuset as at most Scandinavian hospitals the upper limit of MIC group 1 is at present set at 0 25 μg/ml for penicillin and at 1 0 μg/ml for tetracycline

Thus concentrations of penicillin ≥0 25 μg/ml and concentrations of tetracycline ≥1 0 μg/ml in sinus secretions are defined as adequate concentrations In the assay procedures the amount of sinus mucosa in the antibiotic determinations was expressed in grams and not in milliliters Thus in maxillary sinus mucosa concentrations of penicillin ≥0 25 μg/g and concentrations of tetracycline ≥1 0 μg/g are defined as adequate concentrations This is justified as the density of body tissues is ≈1

RESULTS

Antibiotic concentrations in sinus secretion after a single dose

Penicillin was given in a single dose to 14 patients representing 17 diseased maxillary sinuses

An oral dose of 0 8 g penicillin V resulted in concentrations of 0 1 μg/ml in the secretions of 3 sinuses In another 2 sinuses measurable concentrations were not obtained i e concentrations <0 1 μg/ml

After an injection of 1 0 MU (million units) benzylpenicillin the concentrations achieved in the secretions of 5 sinuses was 0 1 1 ? μg/ml

An injection of 1 2 MU procaine benzylpenicillin resulted in concentrations of 0 2–3 6 μg/ml in the secretions of 4 sinuses In another 3 sinuses measurable concentrations were not obtained in the secretions i e concentrations <0 1 μg/ml

Tetracycline was given in a single dose to 13 patients representing 16 diseased maxillary sinuses

An oral dose of 0 5 g tetracycline resulted in concentrations of 0 5–2 7 μg/ml in the secretions of 9 sinuses In another 3 sinuses measurable concentrations were not obtained in the secretions i e concentrations <0 3 μg/ml

After an injection of 0 25 g tetracycline concentrations of 0 5–0 8 μg/ml were achieved in the secretions of 3 sinuses In one sinus no measurable concentration was obtained in the secretion i e concentration <0 3 μg/ml

Antibiotic concentrations in sinus mucosa after a single dose

Penicillin was given in a single dose to 27 patients representing the same number of investigated maxillary sinuses

An injection of 1 0 MU benzylpenicillin resulted in concentrations of 2 0–17 2 μg/g in the mucosas of 10 sinuses The mucosa/serum concentration ratios ranged from 0 3–2 7

After an injection of 1 2 MU procaine benzylpenicillin no measurable concentrations were obtained in the mucosas of 3 sinuses i e concentrations <0 2 μg/g In the mucosas of the remaining 14 sinuses concentrations between 0 3–3 0 μg/g were observed The mucosa/serum concentration ratios ranged from <0 06–1 3

Tetracycline was given in a single dose to 6 patients representing the same number of investigated maxillary sinuses The concentrations in the mucosa of 5 sinuses ranged from 0 8–3 6 μg/g In the remaining sinus mucosa a measurable concentration was not observed i e concentration <0 3 μg/g The mucosa/serum concentration ratios ranged f <0 2–1 7

Penicillin and tetracycline were

taneously as single doses to 14 patients representing the same number of investigated sinuses.

After the administration of 1.0 MU benzylpenicillin intramuscularly and 0.5 g tetracycline orally to 5 patients, the serum concentrations were followed by repeated blood sampling until the sampling of sinus mucosa 4.5–6.5 hours later. The concentrations of penicillin in the sinus mucosas were close to the serum concentrations at the time of sampling but rather low compared to the maximal serum concentrations. The concentrations of tetracycline in the sinus mucosas were always above the serum concentrations at the time of sampling, which in most of the cases meant concentrations close to or higher than the maximal serum concentrations.

Procaine benzylpenicillin 1.2 MU and tetracycline 0.25 g were given to 9 patients. The mucosa/serum concentration ratios for penicillin at the time of sampling was <0.2–1.0. The corresponding ratios for tetracycline were 0.8–1.9. Statistical analysis showed that the ratios for tetracycline were significantly higher than those for penicillin. The probability for no difference was less than 1% (0.1%$<p<$1.0%).

...otic concentrations in sinus ... and corresponding sinus ...cosa after a single dose

In 15 of 47 surgically treated patients the maxillary sinuses contained sufficient amounts of secretion to enable antibiotic assays. Measurable concentrations of penicillin (≥0.2 μg/g) was found in the mucosa in 12 of 15 maxillary sinuses of patients treated with this antibiotic alone or in combination with tetracycline. Measurable concentrations of tetracycline (≥0.3 μg/g) was found in the mucosa of all the 4 maxillary sinuses of patients treated with tetracycline in combination with penicillin. These concentrations of penicillin and tetracycline in the mucosas invariably exceeded the concentrations in the corresponding sinus secretions.

Antibiotic concentrations in sinus secretion after repeated doses

Penicillin V. 1.6 g a day in divided doses was given orally to 36 patients for 2–5 days. Adequate concentrations (≥0.25 μg/ml) were found in 27 of a total of 60 secretion samples (i.e. 45%) obtained from these patients. By statistical analysis (χ^2-test), it was found that the concentrations of penicillin were significantly lower in purulent than in mucous sinus secretions. The probability of no difference was less than 0.1% ($p<$0.1%).

Tetracycline, 1.0 g a day in divided doses was given orally to 32 patients for 2–5 days. Adequate concentrations (≥1.0 μg/ml) were found in 50 of a total of 54 secretion samples (i.e. 93%) obtained from these patients. By statistical analysis it was found that the concentrations of tetracycline were significantly lower in purulent than in mucous sinus secretions. The probability of no difference was less than 5% (1%$<p<$5%).

Comparison between antibiotic concentration and bacterial growth in sinus secretion

Penicillin V, 0.4+0.4+0.8 g a day, was given to 17 patients representing 17 diseased maxillary sinuses. Sampling of sinus secretion was performed prior to and then after 2–3 days and 4–5 days of treatment. Bacterial species isolated prior to treatment as well as penicillin concentrations in the secretions, and bacterial growth in the samples after onset of treatment are given in Table I. Pneumococci and Haemophilus influenzae were the most common findings. No change from one bacterial species to another was observed during treatment. In 9 of 17 samples of sinus secretion aspirated 2–3 days after onset of treatment, the concentrations of penicillin were less than 0.25 μg/ml. Bacterial growth was demonstrated in 7 of these 9 samples. In 3 of 8 samples of sinus secretions with concentrations equal to or above 0.25 μg/ml bacterial growth was present.

Samples of sinus secretions of 10 sinuses were aspirated after 4–5 days of treatment. In

Table I *Bacterial growth and concentrations of penicillin in sinus secretions of 17 diseased maxillary sinuses of 17 patients treated with 0 4+0 4+0 8 g penicillin V per day*

First sample second sample and third sample denotes sinus secretion aspirated prior to 2–3 days and 4–5 days after onset of treatment respectively Bacterial growth after treatment is indicated by + (demonstrable) and − (not demonstrable) Concentrations of penicillin equal to or above 0 25 μg/ml are defined as adequate concentrations

First sample	Second sample		Third sample	
Isolated bacterial species	Conc μg/ml	Bact growth	Conc μg/ml	Bact growth
Pneumococci	<0 25	+	0 6	−
Pneumococci	<0 25	+		
Pneumococci	<0 25	+	<0 25	−
Pneumococci	<0 25	−		
Pneumococci	<0 25	+	0 3	−
Pneumococci	<0 25	−	0 6	−
Pneumococci	0 4	+	0 9	−
Pneumococci	0 6	−	0 5	−
Pneumococci	1 1	−	0 7	−
Pneumococci	1 2	−		
H influenzae	<0 25	+	<0 25	+
H influenzae	<0 25	+		
H influenzae	0 9	−		
β streptococci Group A	9 3	+		
Anaerob streptococci	<0 25	+	<0 25	+
Anaerob streptococci	0 5	+	0 7	−
None demonstrable	0 5	−		
Concentration of penicillin	<0 25	≥0 25	<0 25	≥0 25
Number of secretions	9	8	3	7
Number of secretions with bact growth	7	3	2	0

Table II *Bacterial growth and concentrations of tetracycline in sinus secretions of 14 diseased maxillary sinuses of 13 patients treated with 0 25 g tetracycline 4 times per day*

First sample second sample and third sample denotes sinus secretion aspirated prior to and after 2–3 and 4–5 days of treatment respectively Bacterial growth after treatment is indicated by + (demonstrable) and − (not demonstrable) Concentrations of tetracycline equal to or above 1 0 μg/ml are defined as adequate concentrations

First sample	Second sample		Third sample	
Isolated bacterial species	Conc μg/ml	Bact growth	Conc μg/ml	Bact growth
Pneumococci	0 7	+	1 2	−
Pneumococci	1 5	+	2 4	−
Pneumococci	1 5	+	2 4	−
Pneumococci	1 5	+	4 8	−
Pneumococci	2 4	−		
Pneumococci	2 4	−	2 4	−
Pneumococci	3 0	−		
Pneumococci	7 0	−		
H influenzae	2 4	−	2 4	−
H influenzae	2 4	−		
β streptococci Group A	1 8	+	3 0	+
Anaerob gr neg rods	0 5	+		
None demonstrable	4 5	−		
Concentration of tetracycline	<1 0	≥1 0	<1 0	≥1 0
Number of secretions	2	12	0	7
Number of secretions with bact growth	2	4	0	1

2 of 3 samples, with concentrations of penicillin less than 0 25 μg/ml bacterial growth was demonstrated No bacterial growth was present in 7 samples of sinus secretions where the concentrations of penicillin were above 0 25 μg/ml

Tetracycline 0 25 g 4 times a day was given to 13 patients representing 14 diseased maxillary sinuses Sampling of sinus secretion was performed prior to and after 2–3 and 4–5 days of treatment Bacterial species isolated prior to treatment as well as tetracycline concentrations and bacterial growth of the samples after onset of treatment are given in Table II Pneumococci and Haemophilus influenzae were the most common findings No change from one bacterial species to another was observed during the treatment In 2 of 14 samples of sinus secretions the concentration of tetracycline was less than 1 0 μg/ml after 2–3 days of treatment Bacterial growth was present in both of these samples In 4 of 12 samples of sinus secretions with concentrations above 1 0 μg/ml, bacterial growth was demonstrable

The concentration of tetracycline was above 1 0 μg/ml in all samples of sinus secretions aspirated after 4–5 days of treatment In one of these secretions, bacterial growth was present

DISCUSSION

Bacteriological studies have shown that most maxillary sinusitis are caused by bacteria Despite this fact, clinical trials have not established that antibiotic therapy improves the healing process As in these studies the antibiotic concentrations in serum, sinus secretion and sinus mucosa were not controlled, the results are not conclusive as to whether antibiotic treatment per se has no, or only a slight effect, or whether the antibiotic concentrations at the site of infection were too low to be effective

An international collaborative study of antibiotic sensitivity testing recommends the categorisation of bacteria in four groups 1–4, in decreasing antibiotic sensitivity (Ericsson & Sherris 1971) Using this classification the clinician will thus choose an antibiotic for treatment to which the bacteria are fully sensitive, i e the symbol of group 1 is marked for the antibiotic Maximal antibacterial effect, however, is only achieved if the bacteria are exposed to antibiotic concentrations at least equal to the MIC value (minimal inhibitory concentration) The antibiotic therapy instituted must therefore result in concentrations at the site of infection which at least equal the [illegible] MIC limit for bacteria categorized in [illegible] 1 This report defines concentrations [illegible] that magnitude as *adequate antibiotic concentrations* It may well be objected that this definition of adequate antibiotic concentrations implies antibiotic concentrations at the site of infection far exceeding the MIC values for the bacteria frequently occurring in maxillary sinus infections i e pneumococci streptococci and *H influenzae* (Walter & Heilmeyer, 1969) However the active non protein bound fraction of the antibiotics will vary with the protein content in the secretion and the tissues i e be dependent on the purulence in the secretion and the inflammatory oedema in the mucosa (Verwey & Williams, 1962, Robinson & Sutherland 1965 English, 1967, Kunin, 1967) It must also be remembered that in order to produce a bactericidal effect, the penicillin concentration has to exceed the minimal inhibitory concentration (MIC) 2–20-fold (Eagle & Musselman 1948) Concerning tetracycline there is evidence that high concentrations cause a steeper fall in the viable count of bacteria than do low concentrations (Hamburger & Carleton 1957) These facts imply that the concentrations of antibiotics at the site of the infection must be well above the MIC value if a maximal antibacterial effect is to be achieved

After a single, ordinary clinical dose of penicillin or tetracycline, the concentrations in sinus secretion and mucosa were unexpectedly low in many cases, or not even measurable After repeated doses of penicillin the concentration in the secretion of 55 per cent of the sinuses could still not be regarded as adequate, according to the definition used After repeated doses of tetracycline, the concentration was, however, adequate in the secretion of 93 per cent of the sinuses Further, it was shown that the concentrations of penicillin and tetracycline were significantly lower in purulent than mucous secretions As a purulent secretion indicates a more severe infection than does a mucous secretion (Boyd 1953) the results imply that the probability of achieving adequate concentration is less in cases of maxillary sinusitis with severe inflammatory reaction Thus it is to be presumed that insufficient antibiotic concentrations at the site of infection can be an important cause of therapeutic failure This is the most probable explanation to the findings of Strong & Tonkin (1951) that penicillin sensitive bacteria survived in sinus secretion despite treatment for many days with penicillin

The mucosa/serum concentration ratios of tetracycline were significantly higher than those of penicillin, when the drugs were administered in combination For tetracycline, the mucosa/serum concentration ratios were with few exceptions ≥ 1 Thus, serum concentrations of 2–3 µg/ml, which is well achieved by normal clinical doses (Walter & Heil

Heilmeyer, 1969) will in most cases result in an adequate concentration in the mucosa. In contrast, the mucosa/serum concentration ratio for penicillin was often rather low, in one case <0.06. However, high serum concentrations (5 μg/ml or more) were invariably associated with adequate antibiotic concentrations in the mucosas. In general clinical use, these observations imply that when selecting an antibiotic for the treatment of maxillary sinusitis, the penetration capacity of the drug has to be considered when deciding the dosage and method of administration.

The results of the present study show that in clinical investigations the antibiotic concentrations at the site of infection have to be controlled so as to exclude the possibility of inadequate concentrations causing therapeutic failures. The antibiotic concentration in the sinus mucosa, however, can be determined only by analysis of the antibiotic content in samples of sinus mucosa obtained in connection with surgical treatment. This type of antibiotic control is, thus, less suitable in general clinical studies. However, analysis of the results show that the antibiotic concentration in the sinus mucosa is always higher than the concentration in sinus secretion. This implies that an adequate concentration in sinus secretion is synonymous with an adequate concentration of the sinus mucosa. Therefore an adequate control of antibiotic therapy in maxillary sinusitis can be considered achieved when the concentrations in serum and sinus secretion have been determined.

In the clinical studies so far performed it was sought to assess the antibiotic effect in the treatment of maxillary sinusitis from the disappearance of one or more clinical signs of the sinusitis. For instance, the number of days of illness (Lumio, 1956, Reynolds et al., 1964), the inflammatory swelling of the sinus mucosa determined by X-ray examination (Axelsson et al., 1970, 1971, 1973) and the patency of the sinus ostium (Rantanen & Arvilommi, 1973) have been used as parameters. In most of the studies, however, the retention of sinus secretion in the maxillary sinus registered as the number of necessary sinus irrigations, has been the parameter used in the evaluation of antibiotic effect (Kortekangas, 1964, 1965, Jeppesen & Schaldemose, 1970, Jeppesen & Illum, 1972).

However, the anticipated and specific effect of antibiotic treatment is the elimination of the bacteria from the sinus. Thus, adequate control of the effect can be attained only by the analysis of bacterial growth in the sinus prior to and after treatment. In the present study, those patients treated with penicillin only or tetracycline only for 2–5 days were subjected to this control. Bacterial growth was demonstrable in almost all sinus secretion samples obtained from these patients before treatment. Pneumococci and Haemophilus influenzae were the bacterial species most frequently isolated, which is in accordance with previous bacteriological findings (Nylen et al., 1972, Axelsson & Brorson, 1972). After treatment with repeated doses of either penicillin or tetracycline, the bacteria were eliminated already after 2–3 days from most secretions with antibiotic concentrations regarded as adequate according to the definition used. In contrast, bacterial growth was still present in most of the secretions with concentrations that could not be regarded as adequate for treatment. As the secretion is produced by and lies in direct contact with the sinus mucosa, sinus secretion can be regarded as a representative sample in the control of bacterial presence not only in the sinus secretion but also in the sinus mucosa. Thus, the reported results imply that the specific and anticipated effect of antibiotic treatment, which is the elimination of the bacteria from the sinus, may be achieved in most cases of maxillary sinusitis. However, the prerequisite is that concentrations obtained at the site of infection are at least equal to the upper limit of MIC bacterial sensitivity group I, in the present study defined adequate concentrations.

Sinus secretion could be aspir.
eral maxillary sinuses despite t

analyses showing that the bacteria had been exterminated from the sinus This establishes that the retention or pathological production of sinus secretion may continue or cease only gradually after the specific effect of antibiotic treatment has been achieved The registration of the number of sinus irrigations necessary can therefore not be regarded as a suitable parameter in the assessment of specific antibiotic effect

The implication of the present results is that the use of parameters not suited to their purpose may be one important source of erroneous results in the evaluation of antibiotic effect in the treatment of maxillary sinusitis A reliable evaluation of the antibiotic effect, however, is achieved by determinations of the antibiotic concentrations and bacterial growth in the sinus secretions

ZUSAMMENFASSUNG

Der antibakterielle Effekt antibiotischer Behandlung bei Kieferhohlenentzundung ist durch eine Untersuchung der Beseitigung der Bakterien in der Kieferhohle bei einer Behandlung mit Penicillin und Tetracyclin bestimmt worden Da adaquate antibiotische Konzentrationen Voraussetzung fur einen maximalen antibakteriellen Effekt d wurden die antibiotischen Konzentrationen kon liert

antibiotischen Konzentrationen wurden anhand Kieferhöhlensekreten und/oder den Schleimhauten on 113 Patienten mit Kieferhohlenentzundung bestimmt die mit einmaliger Dosis oder mit wiederholten Dosen Penicillin oder Tetracyclin oder beider Antibiotika in Kombination behandelt worden waren Bei 30 Patienten die entweder mit Penicillin oder Tetracyclin behandelt worden waren wurde das bakterielle Wachstum im Kieferhohlensekret vor und nach der Behandlung kontrolliert

Das Ergebnis dieser Untersuchung war dass ubliche klinische Dosen Penicillin und Tetracyclin wenn auch nicht ausnahmslos antibiotische Konzentrationen in den Kieferhohlensekreten und schleimhauten aufweisen die für die Behandlung als geeignet angesehen werden können d h fur Penicillin ≥0 25 μg/ml und für Tetracyclin ≥1 μg/ml Weiterhin konnte klargestellt werden dass es zur Sicherstellung des therapeutischen Erfolgs notwendig ist dass adaquate antibiotische Konzentrationen im Kieferhöhlensekret und in der Schleimhaut vorliegen Wenn die Penicillin oder Tetracyclinkonzentrationen in den Sekreten nicht als fur die Behandlung angemessen angesehen werden konnten zeigte sich in den Sekreten der meisten Kieferhohlen ein fortgesetztes bakterielles Wachstum Dagegen wurden die Bakterien in den meisten Fällen mit als adäquat beurteilten Konzentrationen beseitigt

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THE ROLE OF PSYCHIC FACTORS IN PATIENTS WITH ALLERGIC RHINITIS

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Abstract Psychosomatic studies were made in 128 patients with allergic rhinitis After the allergic and clinical anamnesis the patients were divided into two groups those ill with hay fever (91 patients) and those suffering from perennial allergic rhinitis (37 patients) The assessment of the role of psychogenous factors in both varieties of allergic rhinitis was the main purpose of the investigation The investigation showed that psychogenous factors are practically of no importance in hay fevers Their role is great however when perennial allergic rhinitis is involved

The role of immunological mechanisms (Dolo- 1974) is decisive in allergic phenomena , the importance of other factors especially of psychic character is almost equally considerable (Dorfman 1970 Sanger 1970) In some patients they can act as stimuli releasing the disease symptoms Emphasis is laid on the fact psychic stress in people genetically predisposed to allergic diseases may produce in them symptoms difficult to control (Sanger 1970)

The earlier researches point to the weight of emotions which influence the course of hay fevers (Dunbar 1954 Hamilton 1955 Haughton 1967 Wilson 1948) Some observations may lead us to the conclusion that psychic stimuli aggravate the symptoms of hay fever In one of the experiments patients were interviewed in a room containing some stable quantity of allergen to which they were sensitized During the anamnesis dealing with different conflict situations in which the patients have been involved the symptoms of hay fever were visibly increased (Holmes et al 1951) It was proved in number of inquiries that psychic stimuli can produce symptoms of hay fever Some patients allergic to a particular genus of flowers suffer an onset of hay fever at the mere sight of imitations of these flowers (Hamilton 1955)

MATERIAL AND METHOD

One hundred and thirty randomly selected patients (65 men 65 women) with allergic rhinitis were examined The ages of the patients ranged between 11 and 64 Most of them (120) were more than [illegible] years old with 97 between 20 and 50 years of age Ninety seven patients lived in Warsaw 20 in other towns and 13 were country people Two patients had to be excluded from the investigation because their allergological anamnesis proved to be unsatisfactory The rest were divided into two groups according to the results obtained in the anamnesis and the clinical examinations The patients with hay fever 91 in all were comprised the first and those suffering from perennial allergic rhinitis (37 cases) the other group

All the patients were subjected to psychosomatic examinations These consisted of psychosomatic anamnesis estimation of the

psychic condition of the patients, and drawing up of an inventory of the patient's personality Moreover some of the ill were put through electroencephalographic tests All the examined were subjected to allergic examinations In some of the patients the level of IgE in blood serum was singed means by radio-immunological assay (J125)

Statistical analysis of the obtained data was carried out by the following methods (*a*) by testing the differences in proportion in two different populations, and (*b*) χ^2 tests with the Yates correction

The aim of the experiment was the comparison of the role of psychic factors in the course of hay fever and perennial allergic rhinitis The investigation is part of the research into allergic rhinitis

RESULTS

Our examination shows that the transmission of hereditary allergic factors was similar among the closest members of the patient's family On the other hand it was a little more frequent among the relatives of the patients with hay fever (48%), than among the relatives of those with perennial allergic rhinitis (43%)

In order to assess the role of psychic factors in the course of hay fever and perennial allergic rhinitis, each case was analysed in two respects One aspect concerned those moments in the past which were essential in the formation of the patient's personality with special consideration of the situations causing frustrations, while the other concerned the present situation of the patient

Factors which are essential for the formation of the personality are the attitude of the parents, or foster-parents, to the child, mutual attitude of the parents (foster-parents), loss of one of the parents (foster-parents) or both Most of the patients i e 93 in the whole examined group were brought up by their parents only with the mother bearing the main burden of upbringing In 12 cases, the grandmother played a similar role, participating in the raising of the child to the same degree as did the mother The remaining patients in the group, i e 4 persons, were brought up by other people or in an orphanage.

For the normal development of a child's personality, a proper attitude of the parents (foster-parents) to the child is very important What is meant here by "proper attitude" is, among other factors, acceptance of the child, reasonably mild and consistent methods of raising it, the same treatment of all the children

Mutual attitudes of parents and children (i e patients) were estimated according to a two-level scale Following the division of the patients into the two groups, the attitudes of the parents to the patients were examined in 30 patients with perennial allergic rhinitis, and 72 with hay fever Assuming the differences on the statistically significant level ($P<0\ 001$) it was found that cases of faulty relations with the father were more numerous in the group of patients with perennial allergic rhinitis (9 patients, 39%), than in the group of hay fever patients (10, 13 8%) The mother (foster-mother) – *child relations were examined in the* group of 90 persons with hay fever, and 32 with perennial allergic rhinitis It appeared that the latter also had more faulty relations with the mother (foster-mother), than those with hay fever (12, 37 5% vs 19, 21 1% with the adoption of the same statistically significant level)

Mutual attitudes of the parents towards each other are also very essential in the formation of an emotional climate The analysis of mutual attitudes of the parents (foster-parents) was made in the group of 90 hay fever patients and 31 with perennial allergic rhinitis Seven patients brought up by one parent only or in the orphanage were excluded from the investigation The conclusion reached was that strained relations between the parents were more numerous in the case of patients with perennial allergic rhinitis (11, 35 5%), than

when the parents of hay fever patients were involved (14, 15.5% with $P<0.001$).

Disintegration of family for various reasons, e.g. death, departure of one or both parents, produces psychic stresses frequently causing strong anxiety reactions. Family disintegration was more frequent in the childhood and early youth of patients with perennial allergic rhinitis (16, 43.2%) than in the case of hay fever patients (21, 23% with $P<0.001$). The most frequent reason for family disintegration was the death of the father (22 patients), mother (4 patients) and loss of both parents (one instance). Divorce or separation caused the loss of full family in the remaining 10 cases.

To estimate the behaviour of our patients in different social situations, their family relations, profession and education were analysed. The percentage of the patients remaining married, with the exception of 10 persons below 18, was similar in both groups and reached 64.6% (53 patients) in the hay fever group and 66.3% (26 patients) in the group of those with perennial allergic rhinitis. No considerable deviations were found in the frequency of divorces and separations in the group of hay fever (7) and perennial allergic rhinitis patients (3). Three patients who had ... their spouses and did not marry again were found only in the group of patients with perennial allergic rhinitis. Most of the patients in the whole group were professionally engaged or studied (115 in all) and in this respect, no essential differences were discovered between the two groups.

Though from a formal standpoint no considerable differences were found between both groups, distinct qualitative variations were brought to light with respect to their adaptability to the conditions of life. Patients with perennial allergic rhinitis were more exposed to frustrations and experienced conflict situations more frequently when fulfilling their duties. It was most evident in the situations when strong emotional union was required. The analysis of the conjugal life revealed the fact that patients suffering with perennial allergic rhinitis had less satisfaction than those with hay fever (10, 52.6% vs. 15, 28.3%, with $P<0.001$). The former were also more dissatisfied with their sexual life (42.8% vs. 18%, with $P<0.04$).

By analogy the patients with perennial allergic rhinitis were more exposed to conflict situations (23%) than the hay fever patients (5.3%, $P<0.05$). More frequent conflict situations among people with perennial allergic rhinitis resulted in more numerous neurotic disturbances in this group of patients (40.5% vs. 11%).

In order to observe the influence of psychogenous factors on the course of the allergic disease, we examined the frequency of conflict situations preceding the appearance of the symptoms of allergic rhinitis as well as direct influence of various emotions on the course of the disease. The examination showed marked differences between both groups. Twenty-four (64.8%) patients were in conflict situations during the period the catarrh symptoms were visible. On the other hand those with hay fever suffered, in the same period, from remarkably fewer tense psychic stresses (16, 17.5%, with $P<0.001$). Greater importance of psychogenous factors in the course of perennial allergic rhinitis was reflected in more frequent influence of psychic stresses on the symptoms of catarrh. This was observed in 9 patients suffering with perennial allergic rhinitis and in 5 with hay fever.

We should emphasize the fact that symptoms of catarrh were reduced in 3 hay fever and 2 perennial allergic rhinitis patients under various psychic stresses.

DISCUSSION

One of the main issues of our examination is the conclusion concerning the role of psychic factors which is different in perennial allergic rhinitis and in hay fever. In the former the importance of psychogenous factors is great

In the case of hay fever, as it seems their influence is only slight Both, facts from the biographies of the ill and the actual situation of the examined, which is often the cause of long lasting stresses, point to it conspicuously

The formation of the patients' personalities in those ill with perennial allergic rhinitis took place in much less favourable conditions in their childhood and early youth than the formation of personalities of the hay fever patients The former were also more frequently in a state of frustration due to the disintegration of their families ($P<0\ 001$) The same group had faulty relations with their parents or foster parents ($P<0\ 001$)

The lack of proper conditions for the formation of adult personalities self dependent and resourceful in different situations in life is the result of repeated conflict circumstances in the childhood and early youth of the perennial allergic rhinitis patients It explains to a large degree why those persons could not manage in different conflict situations in their adult life In such situations their only reactions were of neurotic or psychosomatic character The conflicts become manifest when great emotional engagement is involved It also follows from our investigation that patients with perennial allergic rhinitis who were married had more conflicts in their conjugal lives with the spouses (52 6%, $P<0\ 001$) and less sexual satisfaction (42 1% $P<0\ 04$) than those with hay fever The former experienced more frustrating situations ($P<0\ 05$) than those in the other group As compared with the hay fever patients those with perennial allergic rhinitis had many more neurotic disturbances (40 5% of those examined)

The importance of emotions in the course of psychosomatic disease may be confirmed by, among other things the temporal connection between the action of the circumstances causing frustration and the appearance of the symptoms on the one hand and the direct perceptible impact of psychic stresses on the course of the illness on the other hand In the examined varieties of allergic rhinitis we found distinct differences in the appearance of conflict situations preceding the emergence of allergic disease symptoms They were found in 64 8% patients with perennial allergic rhinitis and in 17 6% patients with hay fever ($P<0\ 001$) Also the direct impact of psychic stresses on the disease symptoms in those with perennial allergic rhinitis was much greater (24 3%) than in those with hay fever (5 5%, $P<0\ 01$)

Consequently, our investigations indicate that emotions in perennial allergic rhinitis patients influenced the appearance of the disease symptoms and were of importance in the further course of the illness in a considerable number of the patients It permits us to place perennial allergic rhinitis among the psychosomatic diseases

Our observations concerning the negligible importance of psychogenous factors in the course of hay fever contrast sharply with the results of some, especially earlier, researches in which emotions were given the priority in the pathogenesis of hay fever Such views were also confirmed by some reports discussing the results of the treatment by psychosomatic methods Haughton (1967) was successful in his use of hypnosis in the treatment of hay fever, by desensitizing his patients "psychologically

It seems that some of the above mentioned observations pointing to the great role of emotional factors in hay fever might have arisen from unsatisfactory materials often limited to only a few cases psychosomatically selected, with consecutive analysis Wilson (1948), Dunbar (1954) They may also be due to differing methods of investigation

CONCLUSIONS

1 *Neurotic disturbances* are often found in patients with perennial allergic rhinitis (40 5%) but are rare in those with hay fever (11%)

2 Psychic factors in the course of hay fever are of minor importance Their role is much

3 Perennial allergic rhinitis can be included among the psychosomatic diseases because of the role of emotions in pathogenesis of the symptoms

4 The treatment of perennial allergic rhinitis requires psychotherapy and psychopharmacotherapy as well as classical cure

ZUSAMMENFASSUNG

Einfluss des psychischen Faktors bei Kranken mit allergischem Nasenkatarrh Es wurden psychosomatische Untersuchungen an 128 Patienten mit allergischem Nasenkatarrh durchgeführt Aufgrund der allergologischen Befragung und der Gesamtheit der ausserlichen Symptome wurden die Patienten in Zwei Gruppen eingeteilt Patienten mit Heuschnupfen (91 Personen) und solche mit saison unabhangigem allergischem Katarrh (37 Personen) Das Ziel der Untersuchungen war die Bewertung des Einflusses der psychischen Faktoren in beiden Abarten des allergischen Nasenkatarrhs Die Untersuchungen haben ergeben dass die psychischen Faktoren keine grossere Bedeutung bei Heuschnupfen haben Dagegen ist dieser Einfluss bei dem von der Saison unabhangigen allergischen Nasenkatarrh bedeutend was bei der arztlichen Behandlung berucksichtigt werden muss

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A CYTOCHEMICAL METHOD OF GRADING THE MALIGNANCY OF SALIVARY GLAND TUMOURS PREOPERATIVELY

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(Received April 17, 1975)

Abstract. Aspiration biopsy is an accurate but still a somewhat controversial method of diagnosing salivary gland tumours preoperatively. During the last few years this method has been more and more accepted dependent on its increasing reliability in diagnosing the different [illegible]

[illegible] ... carcinoma and acinic cell carcinoma—the [illegible] to be ass[illegible] tent whe[illegible] ploid or [illegible] suggest that the [illegible] DNA content may be related to the morphological differentiation and particularly to the inva[illegible]

[illegible] of the tumours preoperatively by aspiration biopsy.

A necessity for an adequate treatment of a tumour is a preoperative knowledge of the clinical malignancy of the tumour. The difference in the general prognosis between the particular salivary gland tumour types is well known (Eneroth, 1971), but the prognosis varies often very much in tumours *within* the same tumour type (Eneroth et al., 1968, Jakobsson et al., 1968, Blanck et al., 1971, Eneroth *et al.*, 1972). *Aspiration biopsy* (fine needle biopsy) is according to our experience a rapid and accurate diagnostic method in evaluating the type of salivary gland tumour preoperatively (Eneroth & Zajicek, 1966, Eneroth & Zajicek, 1969, Zajicek & Eneroth, 1970, Eneroth et al., 1971, Zajicek et al., in press). It is however difficult to grade the malignancy of the individual tumours on a cytological basis, as the most significant morphological criterion in predicting the prognosis of a salivary gland tumour is the behaviour of the tumour towards surrounding tissues such as non-invasive or invasive growth (Eneroth et al., 1966, Blanck et al., 1967, Jakobsson et al., 1968, Blanck et al., 1971, Blanck et al., 1974).

As correct preoperative diagnosis is a necessary prerequisite for an adequate therapy there is a definite need for a method of grading the malignancy of the individual tumours on a cellular level. Our intention has been therefore *to find* a method in grading the malignancy of the individual salivary gland tumours preoperatively on a cytological basis, i.e. not *using the invasive growth as a criterion of malignancy*. As it has been found that different cellular morphological features of malignant salivary gland tumours were not of prognostic importance (Blanck, 1974), our intention has

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Abstract. Aspiration biopsy is an accurate but still a somewhat controversial method of diagnosing salivary gland tumours preoperatively. During the last few years this method has been more and more accepted dependent on its increasing reliability in diagnosing the different types of tumours, but for an adequate treatment it is also important to know the malignancy degree of the individual tumour. Since invasiveness is the most significant morphological criterion in evaluating the prognosis of a salivary gland tumour it has, however, been difficult to grade the malignancy morphologically on a cytological basis. Thus the diagnostic value of the aspiration biopsy method would increase, if beside the morphological determination of the tumour type it was possible to find a cellular criterion which reflects the grade of malignancy. In the present investigation we have studied the nuclear DNA content in non-invasive tumours and invasive tumours. In general invasive tumours were characterized by a higher degree of abnormality with respect to the DNA content than the non invasive tumours. In two tumour types especially studied—mucoepidermoid carcinoma and acinic cell carcinoma—the property of non-invasive growth was found to be associated with a diploid or near-diploid DNA content, whereas invasive growth was associated with a triploid or near triploid DNA content. These data suggest that the nuclear DNA content may be related to the morphological differentiation and particularly to the invasive properties of the salivary gland tumours. Thus cytophotometric DNA analysis of DNA content in the smears from aspirates might be valuable in grading the malignancy of the tumours preoperatively by aspiration biopsy.

A necessity for an adequate treatment of a tumour is a preoperative knowledge of the clinical malignancy of the tumour. The difference in the general prognosis between the particular salivary gland tumour types is well known (Eneroth, 1971) but the prognosis varies often very much in tumours within the same tumour type (Eneroth et al., 1968; Jakobsson et al., 1968, Blanck et al., 1971; Eneroth et al., 1972). Aspiration biopsy (fine needle biopsy) is according to our experience a rapid and accurate diagnostic method in evaluating the type of salivary gland tumour preoperatively (Eneroth & Zajicek, 1966, Eneroth & Zajicek, 1969, Zajicek & Eneroth, 1970, Eneroth et al., 1971, Zajicek et al., in press). It is however difficult to grade the malignancy of the individual tumours on a cytological basis, as the most significant morphological criterion in predicting the prognosis of a salivary gland tumour is the behaviour of the tumour towards surrounding tissues such as non-invasive or invasive growth (Eneroth et al., 1966, Blanck et al., 1967, Jakobsson et al., 1968, Blanck et al., 1971, Blanck et al., 1974).

As correct preoperative diagnosis is a necessary prerequisite for an adequate therapy there is a definite need for a method of grading the malignancy of the individual tumours on a cellular level. Our intention has been therefore to find a method in grading the malignancy of the individual salivary gland tumours preoperatively on a cytological basis, i.e. not using the invasive growth as a criterion of malignancy. As it has been found that different cellular morphological features of malignant salivary gland tumours were not of prognostic importance (Blanck, 1974), our intentio

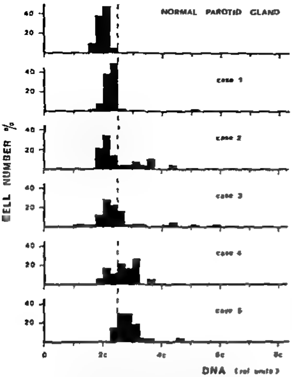

Fig 1 Histograms of nuclear DNA content (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer The cell number in each histogram class is expressed in per cent of the total number of cells analysed Between 50–100 cells were analysed in each preparation Mucoepidermoid carcinomas case 1 2 and 3 were non invasive while mucoepidermoid carcinomas case 4 and 5 were invasive The vertical line represents the upper extreme limit of DNA value of normal parotid cells

been to discover if there are some other criterions of the malignancy at a cellular level

In a previous study (Eneroth & Zetterberg 1974*b*) cytophotometric determinations of nuclear DNA quantity of cells from malignant salivary gland tumours have suggested a relationship between the grade of malignancy and the fraction of tumour cell nuclei containing more DNA than the normal diploid amount

The aim of the present study has been to further explore these observations This was done in a series of malignant salivary gland tumours by analyzing the relationship between the nuclear DNA content and the grade of morphological differentiation especially using "invasive growth" as a principal criterion

If such a relationship could be found, we could preoperatively judge the malignancy of individual salivary gland tumours on a cytological basis

MATERIAL AND METHODS

Twelve cases of malignant salivary gland tumours, treated at the Department of Otolaryngology, Karolinska Sjukhuset, were selected for analysis on the basis of the morphological features—six of the tumours were carcinomas with no definite signs of invasive growth Of the non invasive carcinoma 3 cases were classified as mucoepidermoid carcinoma, one as papillary adenocarcinoma one as acinic cell carcinoma, and one as adenoid cystic carcinoma, whereas of invasive growing carcinomas, 2 were classified as mucoepidermoid carcinoma, 2 as acinic cell carcinoma, and 2 as poorly differentiated carcinoma of a non-classifiable type For microscopic examination of the tumours routine histological technique was employed

Immediately after the surgical removal of the tumours, imprint preparations were made from a freshly cut surface of the tumour tissue Haemacytometer glass slides were used The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and acetone (1 1) for 30 minutes at room temperature and thereafter stored in a refrigerator (+4°C) until the staining was performed

The DNA content of individual cell nuclei was determined after Feulgen staining by absorption measurements in a rapid scanning micro-spectrophotometer at 546 nm (Lomakka, 1965, Caspersson & Lomakka 1970) The optimal condition of the acid hydrolysis in the Feulgen staining procedure was used as described by Eneroth & Zetterberg (1974*b*)

Freshly prepared human lymphocytes from peripheral blood were used as control cells of the staining procedure on each staining occa

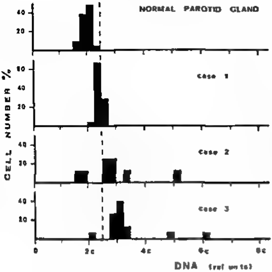

Fig 2 Histograms of nuclear DNA content (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer The cell number in each histogram class is expressed in per cent of the total number of cells analysed Between 50–100 cells were analysed in each preparation Acinic cell carcinoma case 1 was non invasive while acinic cell carcinomas case 2 and 3 were invasive The broken vertical line represents the upper extreme limit of the DNA value of normal parotid cells

sion All measured values were expressed in relation to their corresponding staining control which was given the value 2 C denoting the normal diploid DNA content All DNA values were expressed in such relative lymphocyte units As will be seen from Fig 1 the mean DNA values of the cell nuclei from the normal parotid gland tissue correspond to the normal diploid content (2 0 C) with a variation in the majority of cells between 1 75 C and 2 25 C

Occasionally cells from the normal parotid gland tissue with values up to 2 5 C were recorded The value of 2 5 C was therefore used as an upper limit of the normal diploid DNA content The 2 5 limit is indicated by broken lines in the figures (Figs 1 and 2)

Stromal cells with elongated nuclei lymphocytes and granulocytes could easily be recognized in Feulgen stained preparations and were used as extra control cells (internal control)

RESULTS

As the previously suggested relationship between the fraction of cells with increased DNA values (above the diploid level) and the grade of malignancy might be influenced by the special type of tumour, we have first studied the relationship between the DNA content and morphological differentiation in individual tumours belonging to the same tumour type (Figs 1 and 2)

The nuclear DNA content in five mucoepidermoid carcinomas with various morphological differentiation, mainly with respect to the relation of the tumours to the surrounding tissue, is illustrated by the histograms in Fig 1 Cases 1, 2 and 3 represent non-invasive tumours whereas cases 4 and 5 are invasive tumours

As is evident from the histograms the tumour cell populations in cases 1, 2 and 3 were characterized by a modal DNA value in the diploid region (around 2 C) and by a variable fraction of cells with higher DNA values, occasionally up to the hexaploid level

In contrast to the non invasive tumours (cases 1 2 and 3) the majority of cell nuclei of the invasive tumours (cases 4 and 5) have DNA values in the hypo-triploid or triploid region (between 2 5 C and 3 0 C)

Thus the non invasive mucoepidermoid carcinomas which usually exhibit a low degree of clinical malignancy, have diploid or near-diploid modes in contrast to the invasive mucoepidermoid carcinomas which usually exhibit a high degree of clinical malignancy, which have hypotriploid or triploid modes

We have also studied the nuclear DNA content in three acinic cell carcinomas with various degrees of morphological differentiation, mainly with respect to the relation of the tumours to the surrounding tissues The nuclear DNA content of the cells from a three tumours is illustrated by the

Table I *Frequency of heteroploid cells defined as cells with DNA values above the upper limit (2 5 C) of the normal diploid DNA content in twelve cases of salivary gland carcinoma with a varying degree of invasiveness*

	Non invasive carcinomas						Invasive carcinomas					
Case no	1	2	3	4	5	6	7	8	9	10	11	12
Heteroploid cells %	4	28	32	14	31	39	69	86	75	93	66	91

in Fig 2 Case 1 is a non invasive tumour and cases 2 and 3 are invasive tumours

It is seen from Fig 2 that the majority of cells from the non invasive acinic cell carcinoma (case 1) have nuclear DNA values corresponding to a hyperdiploid amount (2 0 C–2 5 C), whereas the invasive tumours (cases 2 and 3) have a hypo triploid to a triploid modal DNA value (between 2 5 C and 3 0 C)

Thus the situation was similar with the invasive tumours of both mucoepidermoid carcinomas (cases 4 and 5 in Fig 1) and acinic cell carcinomas (cases 2 and 3 in Fig 2) in which the majority of cells have an increased nuclear DNA content corresponding to a near triploid value

Thus for both mucoepidermoid and acinic cell carcinoma modal DNA values corresponding to the diploid or near diploid DNA content were found in non invasive tumours and modal values corresponding to the triploid or near triploid DNA content was the characteristic feature of the invasive tumours

Using the fraction of tumour cells with increased DNA values (>2 5 C) as a gross parameter of cytochemical abnormality a series of 12 malignant salivary gland tumours have also been studied (Table I)

This fraction of cells varies between 4% and 39% in the six non invasive carcinomas—three of them histologically classified as mucoepidermoid carcinoma one as papillary adenocarcinoma one as acinic cell carcinoma and one as adenoid cystic carcinoma In the invasive carcinomas this fraction is considerably increased and varies between 66% and 93% These invasive carcinomas were histologically classified as mucoepidermoid carcinoma (2 cases), acinic cell carcinoma (2 cases) and poorly differentiated carcinoma (2 cases)

DISCUSSION

The varying degree of malignancy both of the many various types of salivary gland tumours and of the individual tumours of the same type has led to a considerable uncertainty with respect to treatment Even if there is a difference in the general prognosis between the different tumour types (Eneroth 1971) it is often a great variation in the grade of malignancy of the individual tumours belonging to the same tumour type Thus in most of the malignant salivary gland tumour types such as acinic cell carcinoma (Seifert 1966 Kleinsasser et al 1967) mucoepidermoid carcinoma (Linell 1948 Woolner et al 1954 Foote & Frazell 1954 Eneroth 1964) adenoid cystic carcinoma (Eneroth et al 1968) and mucous producing adenopapillary carcinoma (Blanck et al 1971) it has been found that the grade of malignancy varied within the same tumour type with relation to the morphological differentiation of the individual tumour

In large series of the different malignant types of parotid tumours such as acinic cell carcinoma (Eneroth et al 1966) adenoid cystic carcinoma (Blanck et al 1967) mucoepidermoid carcinoma (Jakobsson et al 1968) mucous producing adenopapillary

carcinoma (Blanck et al., 1971) and poorly differentiated solid carcinoma (Blanck et al., 1974). It has been found that the degree of cellular polymorphism, the mitotic rate and the lymphoid stroma reaction did not carry much prognostic weight, whereas invasive growth offered a good possibility in prognostic evaluation of the tumours.

As excision biopsy for different reasons must be avoided in the parotid gland (Eneroth 1973) aspiration biopsy according to our experience is the best preoperative diagnostic method in evaluating the type of salivary gland tumour. Even if the morphological details in the cytological smear permit identification of the different tumour types with a very high degree of certainty (Eneroth & Zajicek, 1966, Eneroth & Zajicek, 1969, Eneroth et al. 1971 and Zajicek et al. in press), it is however not possible with this method to decide the grade of malignancy of the individual tumours as the relation of the tumour to surrounding tissue cannot be studied in the cytologic smear.

As a preoperative determination of the grade of malignancy *however* is very important for an adequate therapy, e.g. if preoperative irradiation should be given or not and the extension of the surgical intervention, there is a need for a method of grading the malignancy of individual tumours on a cellular level. Cytophotometric DNA analysis of benign and malignant salivary gland tumours (Eneroth & Zetterberg, 1973, Eneroth & Zetterberg 1974*a* and *b*, Eneroth et al. 1974) have shown that benign tumours such as pleomorphic adenoma and monomorphic adenoma have DNA values identical with those of normal cells from the parotid gland, whereas a fraction of cells from malignant salivary gland tumours showed DNA values exceeding that of normal cells and with a variation according to the type of tumour.

Furthermore, the observations earlier made by *Eneroth & Zetterberg (1974b) suggested a* relationship between the fraction of cell nuclei with abnormally increased DNA values and grade of malignancy of the individual tumour. This idea was further explored in the present study in which the relationship between the cytochemical DNA characteristics and the degree of morphological differentiation was analysed in more detail in a series of malignant salivary gland tumours, especially considering *the criterion of invasive growth*.

The results of the present study indicate that there is a connection between the cytochemical characteristics with respect to the nuclear DNA content and the malignancy of the individual tumour, based on the morphological criterion invasive growth. Thus it was found that non invasive tumours belonging to the same type have a diploid or near diploid DNA content, whereas invasive tumours of the same type have a triploid or near triploid DNA content.

In a series of twelve malignant salivary gland tumours of various types it was found that the fraction of tumour cells with increased DNA values (>2.5 C as a gross parameter of cytochemical abnormality) was considerably more increased in invasive tumours than in non invasive tumours.

Changes in chromosome number and nuclear DNA content have been found to be associated with changes in morphological differentiation in some other tumour types, such as tumours of the urinary bladder (Lamb, 1967, Cooper et al. 1969, Levy et al., 1969, Lederer et al. 1972), cancer of the cervix uteri (Cervenka et al. 1973), prostatic carcinoma (Zetterberg & Esposti, to be published).

These findings support the results from the present study that the nuclear DNA content may be a cellular criterion which reflects the grade of malignancy of the individual salivary gland tumour.

Cytophotometric analysis of DNA content in the cytological smears from individual salivary gland tumours might therefore be a valuable method in grading the malignancy of *the tumours preoperatively* by aspiration biopsy. By a better preoperative diagnosis of the malignancy the adequate treatment of the individual tumours is facilitated.

ZUSAMMENFASSUNG

In der praoperativen Diagnosestellung bei Speichel drusentumoren ist Aspirationsbiopsie *eine sorgfaltige* jedoch noch immer etwas umstrittene Methode Wahrend der letzten Jahre ist diese Methode mehr und mehr akzeptiert worden da die sich in der Diagnose der unterschiedlichen Tumorentypen in zunehmendem Masse als zuverlassig erwiesen hat Jedoch ist es fur eine jeweils adaquate Behandlung zugleich wesentlich den Grad der Malignitat des individuellen Tumors zu kennen *Da Tumorinvasion das signifikanteste morphologische* Kriterium bei der Beurteilung der Prognose von Speichel drusentumoren ist war es jedoch bisher schwierig die Malignitat morphologisch auf zytologischer Basis einzu stufen Der diagnostische Wert der Aspirationsbiopsie Methode wurde daher grosser wenn es neben der morphologischen Bestimmung des Tumortyps moglich ware ein zellulares Kriterium zu finden das den Grad der Malignitat erkennen lasst

In der vorliegenden Arbeit ist der Gehalt an nuklearem DNS in nicht invasiven Tumoren und invasiven Tumoren untersucht worden Allgemein gesehen war fur die invasiven Tumoren charakteristisch dass sie einen hoheren Grad an Abnormitat im Hinblick auf den DNS Gehalt aufwiesen als die nicht invasiven Tumoren Bei zwei speziell untersuchten Tumortypen den Muko epidermoid Karzinomen und den Acinuszell Karzinomen zeigte es sich dass die Eigenschaft des nicht invasiven Tumorwachstums mit diploidem oder im diploiden Bereich liegenden DNS Gehalt assoziiert war wahrend invasives Wachstum mit triploidem oder im triploiden Bereich liegenden DNS-Gehalt gekoppelt war Diese Daten lassen vermuten dass der nukleare DNS Gehalt *mit der morphologischen Differenzierung und im besonderen* mit den invasiven Eigenschaften der Speicheldrusen tumoren in Beziehung steht Eine zytophotometrische DNS Analyse des DNS-Gehalts von aspirierten Probe *ihmen durfte d ther bei der Einstufung der M ilignitat* Tumoren mittels Aspirationsbiopsie praoperativ von rte sein

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SCANNING ELECTRON MICROSCOPY OF FUNGIFORM PAPILLAE ON THE TONGUE OF MAN AND MONKEY

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Abstract The surface topography of the fungiform papillae of homo and monkey was studied by scanning electron microscopy Different methods of specimen preparation were tried Satisfactory preservation was obtained with fixation in formaldehyde or in glutaraldehyde with postfixation in OsO_4 followed by critical point drying Taste pores were found in nearly half of the fungiform papillae Up to 3 pores located in the dorsal surface were observed in a single papilla Most taste pores opened in the form of a rounded crater with a diameter of about 5–7 μm elevated slightly above the surface of the papilla Microvilli could be observed in large bore pores

a scanning electron microscope (SEM) ivid impression can be obtained of the ee dimensional morphology of biological surface structures During recent years this technique has been increasingly employed in the study of different sensory organs Regarding the taste organ SEM studies on several species of animals have been made Thus, Graziadei (1969) examined fungiform papillae in the rat and the frog, as well as taste buds in the lip of fish SEM studies on the taste organ of the frog have also been reported by Shimamura & Tokunaga (1970) and Graziadei & De Han (1971) Scanning electron micrographs of different papillae in rabbit, rat and dog have been presented by Beidler (1969)

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Shimamura et al (1972) were the first to investigate in greater detail the pore of mammalian taste buds The last named authors studied circumvallate and, particularly foliate papillae in the rabbit, and described the occurrence of two types of cellular projections or taste hairs

Scanning electron micrographs have also been used to illustrate the topographical changes which take place during the development of the circumvallate, filiform and fungiform papillae of the rat (Mistretta 1972) and during atrophy of the lingual mucosa of the cat after nerve transection (Mizuno et al , 1973)

So far, the SEM technique has only been used to a very limited extent for the study of the human tongue Filiform and foliate papillae have been examined, at different ages by Skach & Svejda (1972) and Svejda & Janota (1974) Arenberg et al (1969) studied the outer taste pore of fungiform papillae from a human tongue

The present report is concerned with the surface structure of the fungiform papillae of man and monkey as shown by the scanning electron microscope This study is part of a larger investigation on the physiology of the taste receptors of the fungiform papillae, and was prompted by the lack of detailed morphological information in the literature

Fig 1 Monkey (*Cynomolgus*) Fungiform papillae surrounded by numerous filiform papillae Formaldehyde fixation SEM, ×100

MATERIAL AND METHODS

The human material was obtained from dental patients, aged 20–50 years, with a normal tongue mucosa Single fungiform papillae or small pieces of mucosa bearing 2–3 such papillae, were removed under surface, or conductive, anesthesia from the anterior part of the tongue Before the biopsy the tongue was sprayed with Ringer solution

The simian material was taken from young adults of two closely related strains of monkey *Cynomolgus* and *Cercopithecus* The anterior third of the tongue was cut out under general anesthesia and sprayed with Ringer solution Small pieces of mucosa with up to 10 fungiform papillae were dissected out

After removal the specimens were rinsed in several changes of physiological saline for 15–20 min to cleanse the surface from mucinous substances The tissue material was then fixed overnight in 4% formaldehyde in phosphate buffer, pH 7 4 (Lillie, 1948), or, for 30 min in 2% glutaraldehyde in 0 1 M cacodylate buffer, pH 7 4, followed by postfixation for 3 hrs in 2% osmium tetroxide

After fixation the specimens were rinsed in distilled water and dehydrated in graded ethanol or graded acetone Up to this point all processing steps were carried out at room temperature The specimens were dried by the critical point method (Anderson, 1951), using a Model E 3000 drying apparatus (Polaron Equipment Ltd , London, England) charged with carbon dioxide The dry specimens were mounted, with adhesive silv paste, on specimen carriers and coated carbon and gold in an Edwards evaporator in order to prevent cha

Fig 2 Homo A single fungiform papilla is demonstrated covered by a squamous epithelium showing physiological desquamation An invaginat on (*upper right*) and the openings of three taste buds (*arro s*) are visible Formaldehyde fixation SEM ×220

specimens by the electron probe Observa were made using a Cambridge S4 scan electron microscope operated at 18 kV

OBSERVATIONS

The scanning electron microscopic findings were similar in both the human and the simian materials and are therefore presented together

General observations

The fungiform papillae are distributed on the dorsal surface of the tongue They occur sparely on the body of the tongue but are more numerous on the tip and on the lateral margins As shown in Fig 1 each fungiform papilla is surrounded by many filiform papillae

The surface of the papilla is covered by a stratified squamous epithelium (Figs 2–4) The polygonal cells are closely joined together, their borders appear as elevated lines in the micrographs Desquamation of the epithelial cells was regularly observed In some of the fungiform papillae an invagination was found in the center of the dorsal surface (Fig 2)

Taste pores

Taste pores occurred in nearly every second fungiform papilla examined in the scanning electron microscope Up to three pores were observed in a single papilla (Fig 2) The pores were situated on the convex dorsal surface of the papilla without any definite pattern of location

In most cases the pores opened in the form of a rounded crater slightly elevated above the surface of the papilla (Fig 5) The wall of the crater was formed by 3 to 4 squamous epithelial cells lying side by side The diameter

Fig 3 Homo Epithelial lining of dorsal surface of a fungiform papilla Superimposed squamous cells with polygonal ledge like borders are demonstrated The location of rounded or oval nuclei is indicated by the presence of smooth elevations Glutaraldehyde + OsO_4 fixation SEM ×1100

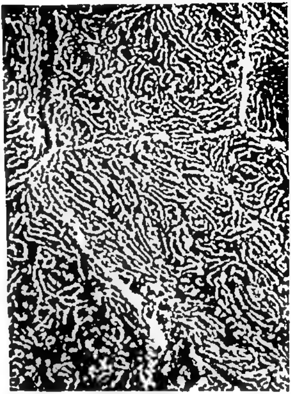

Fig 4 Homo Close up of terraced squamous cells showing microplicae—typical of squamous epithelial cells—and ledge like borders Formaldehyde fixation SEM ×4400

of the opening varied between different taste buds within a range of 1–12 μm (Figs 5–7) most of the taste pores however had a diameter of about 5–7 μm In some instances the free margin of the epithelial cells appeared to bend slightly down into the pore (Figs 5 7) Occasionally an inner pore was found at the bottom of the crater (Fig 6)

In large bore pores microvilli could be observed as shown in Fig 8 The microvilli were irregularly arranged and did not extend to the free margin of the pore Larger club like cellular processes were sometimes noted among the microvilli in the human specimens

DISCUSSION

The preparation of soft biological tissues for scanning electron microscopy is a complex procedure Briefly it involves the following steps (1) removal of extraneous surface matter (2) fixation (3) dehydration and drying and (4) mounting and coating Since the SEM is a relatively new instrument processing techniques are still to a large extent at the trial and error stage no single scheme is suitable for the preparation of all biological samples For reviews of the different technical procedures and of the problems connected with preservation of *in vivo* structure see Boyde & Wood (1969) Marovitz et al (1970) Arenberg et al (1971) and Hollenberg & Erickson (1973) In the present investigation pilot studies were performed to evaluate the suitability of three different fixatives for the preparation of tongue samples Satisfactory fixation was obtained with hyde and with glutaraldehyde

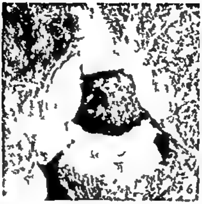

Fig. 5. Homo. Detail from Fig. 2 showing a typical crater-like taste pore. SEM ×1490.

Fig. 6. Monkey (*Cercopithecus*). Epithelial cells are seen to cover partially the inner pore of a taste bud. Formaldehyde fixation. SEM ×1260.

Fig. 7. Monkey (*Cynomolgus*). A very narrow pore of a taste bud is demonstrated. Formaldehyde fixation. SEM ×1490.

postfixation in osmium tetroxide. On the other hand, primary fixation in osmium tetroxide caused shrinkage and cracks in the specimens and was abandoned. Two different methods of drying were also compared, *viz.* critical point drying (Anderson 1951) and freeze drying. Freeze drying was done from 70% ethanol at −35°C over phosphorous pentoxide using the apparatus described by Olson & Ungerstedt (1970). Good preservation was obtained with critical point drying. Freeze-drying led to the development of fissures and, therefore, was not used for the main study.

The taste organ in mammals is located in specialized receptors, the taste buds, which

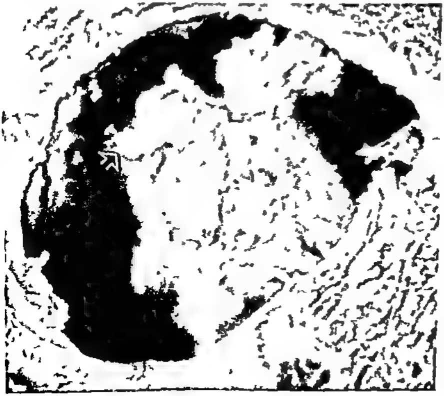

Fig. 8. Homo. Close-up of taste pore. Numerous structures similar to microvilli and a larger club-like cellular process (*arrow*) are visible. Formaldehyde fixation. SEM ×8300.

are found particularly on the tongue In the human tongue taste buds are found mainly in the fungiform and circumvallate papillae A subsequent light microscopic study on the fungiform papillae, made in our laboratory, has shown that taste buds occur in about 40% of the papillae (Arvidson to be published) The taste buds were always confined to the dorsal surface on the papillae but, otherwise, did not show any definite pattern of location Usually, one to three buds were found in a single bud bearing papilla but, occasionally, up to some 20 taste buds could be discerned In the main these findings are in accordance with the present SEM observations, which, moreover, indicate a close similarity in surface structure between human and simian fungiform papillae More than three taste pores were, however, not observed in the present material, presumably due to the smaller number of papillae examined

Another finding with the SEM was the occurrence unrelated to the presence of taste buds of an invagination in the central part of the dorsal surface of some of the fungiform papillae Such invaginations were noted also in the above mentioned light microscopic study Hence, it seems most likely that these invaginations represent a normal feature in the surface morphology of the fungiform papillae and are not to be regarded as artifacts caused by bulk shrinkage during the processing for the SEM

Most of the taste pores opened in the form of a rounded somewhat elevated crater This is in accordance with previous observations in rats (Graziadei 1969) and rabbits (Shimamura et al 1972) The human and simian taste pores had a diameter of about 5–7 μm the corresponding figures for rats and rabbits were given as 1–2 μm and up to 4 μm respectively In the present material there were however considerable differences in the shape and size of the pore opening even in the same tongue To some extent these differences could be caused by fixation artifacts Nevertheless it seems reasonable to assume that the differences observed are primarily due to variations in the arrangement of the outermost epithelial cells during the continuous process of desquamation and cell renewal Whether or not short term variations in pore diameter occur in response to changes in the local environment of the oral cavity cannot be determined from the present observations

Previous transmission electron microscopic studies on the taste buds of fish frog, rat, rabbit and monkey have demonstrated that microvilli project from the apical surface of the taste receptor cells towards the taste pore (for reviews see Graziadei, 1970, Jeppsson 1969, and Murray & Murray, 1970) In addition the occurrence of larger, finger- or club-like projections among the microvilli has been reported in fish (Hirata 1966, Graziadei, 1969) and rabbit (Murray & Murray, 1967) These findings have been confirmed by later investigations using the SEM Thus, microvilli have been observed in frog (Shimamura & Tokonaga, 1970 Graziadei & De Han 1971), fish (Graziadei 1969) and rabbit (Shimamura et al , 1972) The presence, in the two last-named species of a second larger type of projections has also been verified by scanning electron microscopy The most detailed observations on the microvilli have been made in the frog because of the unique arrangement of its taste organ In this animal the sensory area of the taste bud has the shape of an open disc and is, therefore totally exposed Conversely in the other species investigated the microvilli are contained within the pore and are much more difficult to examine in the SEM In the rat, with its small bore pore and short microvilli it has not been possible to visualize these latter structures by means of scanning electron microscopy The present SEM observations have demonstrated the occurrence not previously shown, of microvilli in human taste buds and confirmed their presence in simian taste buds Furthermore larger cellular projections were not
human taste buds, but were not

the papillae taken from monkeys Nor were such larger projections found in previous studies with the transmission electron microscope in the monkey (Murray & Murray, 1960)

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ZUSAMMENFASSUNG

Die Oberflachentopographie der pilzformigen Papillen der Zunge beim Menschen und beim Affen wurden im Rasterelektronenmikroskop studiert Verschiedene Methoden der Specimen Praparation wurden versucht Zufriedenstellende Ergebnisse wurden mit Fixierung in Formaldehyd erreicht desgleichen mit Glutaraldehyd und Nachfixierung in OsO_4 mit nachfolgender kritischer Punkt Trocknung Geschmacksporen wurden in etwa der Halfte aller pilzformigen Papillen gefunden Bis zu 3 Poren belegen auf der dorsalen Oberflache wurden in einer einzelnen Papille beobachtet Die meisten Geschmackspapillen offneten sich in der Form eines abgerundeten Kraters mit ca 5–7 μm Durchmesser etwas erhaben uber der Oberflache der Papille Mikrovillen konnten in weiter geoffneten Poren beobachtet werden

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DEVIATIONS IN THE ROUTE OF THE GREATER PETROSAL NERVE FOR THE NEEDS OF VIDECTOMY'

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Abstract In a matenal of 76 human skulls the authors have tned to locate the route and shape of the greater petrosal nerve canal and pterygo d nerve canal w th spec al attent on g ven to dev at ons from the typ cal route

Growıng ınterest ın surgıcal cuttıng (neurec tomy) of the greater petrosal nerve has been stımulated by faılures to achıeve permanent cure of a number of rhınologıcal dıseases such as rhınıtıs vasomotorıca and neuralgıa of pterygopalatıne nerve Varıous methods have been used to cure those syndromes (Goldıng Wood 1961 Malcomson 1959 Romesıl 1969) Wıth some methods (Malcomson 1959 Romesıl 1969) a consıderable proportıon of the cases operated on faıled to obtaın the ex pected ımprovement It ıs very lıkely that the faılures were due to varıabılıty of the route of the greater petrosal nerve and pterygoıd nerve canal

The tradıtıonal descrıptıon of the route and topography of those nerves ıs as follows the openıngs of the greater and lesser petrosal nerve canals are located on the anterıor sur face of the petrous part of temporal bone (pyramıs)

The greater petrosal nerve canal ıs longer and wıder and has a more medıal posıtıon It begıns at the genu of the facıal nerve canal It contaıns the greater petrosal nerve whıch comprıses the afferent fibres to the genıculate ganglıon and the petrosal branch of the mıddle menıngeal artery

The lesser petrosal nerve canal contaıns a nerve whıch ıs an extensıon of the tympanıc nerve Extendıng to the top of the pyramıd both canals change ınto grooves at first run nıng parallel to each other and end on the la teral wall of the foramen lacerum

The pterygoıd canal whıch connects the basal part of the foramen lacerum wıth the posterıor wall of the pterygopalatıne fossa breaks through the base of the medıal ptery goıd plate Through the fossa there runs the greater petrous nerve and the deep petrous nerve comıng off the sympathetıc plexus at the ınternal carotıd artery as well as the artery and veın of the pterygoıd canal

The above descrıptıon ıs not satısfactory ın ıts detaıls for the needs of mıcrosurgery ın thıs regıon Because the Otolaryngologıcal Clınıc of the Medıcal School ın Warsaw has started surgıcal treatment of those syndromes and at the same tıme the Instıtute of Gross Anatomy has gathered many bone samples the authors have studıed ın detaıl the route and locatıon of the canals of both nerves

MATERIAL AND METHODS

Seventy sıx skulls have been used ın the The measurements were made wıt callıper wıth nonıus and each tı

Table I

	Results of measurements cm					
	1	2	3	4	5	6
Average	3 08	1 82	1 07	0 43	2 88	2 63
Range	2 20–3 80	1 46–2 [illegible]	0 81–1 33	0 17–1 0	1 40–3 [illegible]	1 30–3 31

of the canals was measured with a copper wire The following features, which in the authors' opinion may be of fundamental importance for the surgical approach to the nerves, have been taken into consideration

1 the distance between the pterygoid groove and the entrance to the pterygoid canal,

2 the length of the pterygoid canal measured from its entrance to the mouth in the pterygopalatine fossa,

3 the distance between the circular entrance and the mouth of pterygoid canal measured in the pterygopalatine fossa,

4 the thickness of the base of the pterygoid apex measured between the bottom of the scaphoid fossa and the centre of the pterygoid canal,

5 the length of the pterygopalatine canal measured between the greater palatal foramen and the postero superior wall of the pterygopalatine fossa at the mouth of the pterygoid canal,

6 the distance between the groove and the bottom of the scaphoid fossa Average results

Table II *Deviations from the normal route of pterygoid nerve in 76 skulls*

Group	Feature	Number of skulls
1	The nerve runs in the sulcus under the thin bone plate or the mucous membrane of the floor of the sphenoid sinus (Fig 1)	5
2	The route of the nerve is in the corpus of sphenoid bone	1
3	The nerve runs ridge like protruding above the floor of the sphenoid sinus (Fig 2)	2
4	The nerve does not run on the skull base but goes in a bony sulcus connecting the opening of its canal on the anterior surface of the pyramid and the mouth of the pterygoid canal (Fig 3)	12
5	The entrance of the canal is covered by the top of the pyramid	1
6	The canal runs obliquely to the extension of the pyramid axis	1

Fig 1 Route of the pterygoid nerve sulcus under the mucous membrane of the sphenoid sinus *1* hiatus canalis facialis *2* foramen ovale *3* foramen lacerum *4* sinus sphenoidalis

It could be found on the surface of the part of the petrous bone in direct proximity of the carotid canal At the top of pyramid the sulcus changed into the pterygoid canal in 16% of the samples examined

Our data have thus confirmed the observations made by P H Golding Wood, who examined the distance between the circular entrance and the mouth of the pterygoid nerve canal in the pterygopalatine fossa and estimated it to be 10–12 mm long On the other hand, the length of the pterygopalatine canal measured from the greater palatal entrance to the mouth of pterygoid nerve canal of the pterygopalatine fossa is on average 2 88 cm

Fig 2 Route of the nerve on the bottom of sphenoid sinus seen as a ridge *1* sphenoid sinus *2* bone ridge

Fig 3 Route of the greater petrous nerve sulcus on the front surface of the pyramid coming directly into the pterygoid canal *1* hiatus canalis facialis *2* f spinosum *3* foramen ovale *4* foramen canalis pterygoideus

of the above measurements are given in Table I

When making the measurements some characteristic deviations from the normal route of greater petrosal nerve sulcus and pterygoid nerve canal were discovered They were divided into six groups as set out in Table II

DISCUSSION

The deviations which have been found explain at least to a certain degree the failures of methods of surgical treatment now in use The diseases of the sphenoid sinuses may produce some disturbances of this nerve One or more of those deviations were observed in 29% of the material examined (see Table II)

ZUSAMMENFASSUNG

Anhand eines 76 menschliche Schadel umfassenden Materials wurden der Verlauf und die Gestalt des Kanals des N petrosus major und des Kanals des N pterygoideus sorgfaltig untersucht Bei 29% der Schadel wurden Abweichungen vom normalen Verlauf festgestellt Die Variabilitat des Verlaufs und der Gestalt der genannten Nervenkanale kann dem Chirurgen grosse Schwierigkeiten bei der Neurektomie des N petrosus major bereiten und nicht selten zum Misserfolge der Behandlung mit einer der ublichen Methoden der Neurektomie fuhren

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THE INFLUENCE OF GUANOXANE ON THE SUBMAXILLARY GLANDS OF RATS

Another Model of Neurogenic Sialoses

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Abstract In extracts of submaxillary glands of rats treated with the guanethidine derivative guanoxane (Envacar®) α amylase activity and protein concentration were measured Both parameters were also determined for the α isoamylase patterns obtained following electrophoretic separation of gland extracts in polyacrylamide gels During the first week of drug application amylase was found to be congested in the glands Compared with untreated animals total amylase concentration and amylase activity per unit protein (specific enzyme activity) were significantly increased in extracts and separated isoenzymes whereas protein concentration remained unaltered The described changes reverted to normal after continued drug application for 3 weeks The possibility is suggested that changes in α isoamylase patterns might eventually be employed for clinical characterization of different types of neurogenic sialosis

Dryness of the mouth and pain in the salivary glands at the beginning of mastication are well known side-effects of many antihypertensive drugs (Wilson, 1965, Bock, 1968, 1970, Schneider, 1973) Their action involves the vegetative nervous system, diminishing the sympathicotonus ; Clonidine, a clinically widely used antihypertensive drug influences the sympathetic cardiovascular regulation centres of the medulla oblongata (Kobinger, 1973), guanethidine derivatives such as guanoxane [2 guanidinomethyl (1 4) benzodioxane] inhibit the transmission of impulses in postganglionic sympathetic nerve endings (Peart & MacMahon, 1964, Davey & Reinert 1965)

The submaxillary glands of rats show morphologic and, especially, enzymatic changes after a 3 week clonidine treatment The changes in the activity of isoamylases persist even after discontinuation of drug application (Chilla et al , 1975) Although we attributed these changes to some kind of deficiency (Karenz") sialosis with concomitant secondary infection, the fact remains that clonidine acts primarily on the vegetative nervous system (Hoefke & Kobinger, 1966) The secretion process in the salivary glands is probably inhibited to some extent in this manner (Onesti et al , 1971)

Since the sialotic alterations in the glands are primarily caused by neurogenic mechanisms, one might speak in this case of a neurogenic type of a sialosis The clonidine-mediated changes in the rat submaxillary glands could then serve as a model of the central type of a neurogenic sialosis Apart from this sialotic form, there exists also the peripheral-vegetative type (Rauch, 1959) Since guanoxane blocks the transmission of sympathetic nerve impulses peripherally, the question arises whether sialotic changes can be demonstrated in the submaxillary glands of rats after application of guanoxane Afte related studies on rat parotid glands (Ar & Chilla, 1975) we presented a mod

Fig 1 Activity staining with Lugol's solution after *electrophoretic separation in polyacrylamide gel (pH 8 9)* of 2 μg protein from submaxillary gland extract Electro phoresis was carried out at 0°C 600 V 60–80 mA Running time 2 5 hrs Besides the two isoamylases visible in the photograph two more isoenzymes were detected by amylase assay in gel sections farther removed from the origin

peripheral vegetative type of a neurogenic sialosis By extending our investigations to the submaxillary glands we hoped to confirm those earlier findings Again, α amylase with its 4 isoenzymes was selected as a suitable para meter for the glands' metabolic activity

MATERIAL AND METHODS

45 female rats (120–160 g, strain Sprague–Dawley NIH/HAN) were obtained from Zentralinstitut fur Versuchstierzucht, D-3 Hannover/FRG They were divided into 5 groups of 7 and 2 groups of 5 animals each and kept under the same conditions (Altromin® standard diet) Groups I–V were given one intraperitoneal injection per day of 7 mg guan oxane sulphate[1] dissolved in 1 ml of 1 mM phosphate buffer (pH 6 1) The remaining 2 groups served as controls and received no injections After a 12 hrs starvation period the rats were killed in ether narcosis Group I was sacrificed after 1, group II after 3 group II after 7, and group IV after 21 days Group V had received guanoxane for 1 week but had not been treated for 3 more weeks before the animals were killed The control groups VI and VII were sacrificed after 7 and 21 days respectively

The submaxillary glands of the left side were removed at once, weighed, and kept *frozen at − 70°C until used Extraction and* electrophoretic separation procedures were performed as specified elsewhere (Chilla et al 1974, 1975) After electrophoresis, 12 gel strips (3 mm wide) were sampled and eluted as described by *Arglebe & Chilla (1975)* and Arglebe et al (submitted for publication) Protein amounts in the gland extracts were determined after Lowry et al (1951) and amylase activity, both in extracts and gel cuttings, with the assay of Street & Close (1956) The gels run for the quantitative determination of isoamylase activities (Fig 1) were charged with 2 μg and those for estima tion of protein in the amylolytically active bands with 100 μg protein from each sample By staining the latter with Coomassie Blue (Supranolcyanin 6B) the location of protein in the gel was revealed (Koenig et al 1970) The peak areas obtained after densitometry at 546 nm (Chilla et al , 1974, 1975) of the protein zones coincident with the site of isoamylase activity were, in the present study, transferred to graph paper Prior to this it had been verified that the paper had the same weight per area unit in all places The circumscribed areals were cut out and weighed, the respec tive weights (mg) are referred to as *relative* protein amounts

Levels of statistical significance for the experimental data were computed using the Mann–Whitney (Wilcoxon) two sample statis tic (Milton, 1964)

RESULTS

After electrophoretic separation of 2 μg pro- tein from the gland extracts, 2 bands of iso-

[1] Pfizer GmbH D 75 Karlsruhe/FRG kindly provided us with a sample of guanoxane sulphate Envacar®)

Table I *Unseparated gland extracts . Protein concentration, total and specific amylase activities*

S C U =Street–Close units Specific amylase activities were first calculated for each individual gland average values shown in column (spec act) of this Table were computed from those data Consequently averages (amylase) divided by averages (protein) do not yield averages (spec act)

Group	Days treated	Days untreated	Amylase activity (S C U /ml extr)	Protein conc (mg/ml extr)	Specific activity (S C U /mg protein)
I (n=7)	1	–	148 (0 1%[b])	46 6	3 220 (0 1%)
II (n=7)	3	–	29 (1%)	48 7	0 632 (2%)
III (n 7)	7	–	144	45 5	3 370
IV (n=5[a])	21	–	17 4	35 5 (5%)	0 517
V (n=6[a])	7	21	3 1 (5%)	40 8	0 078 (10%)
VI/VII (n–10)	–	7/21	7 1	43 5	0 177

[a] 2 rats (group IV) resp 1 rat (group V) died after injection
[b] Difference to control Levels of significance ($p \leq n$ %)

amylases (Fig 1) could always be demonstrated by activity staining with Lugol's solution (Chilla et al , 1975) Evidence for another 2 isoamylase fractions, predominant in parotid gland extracts, could in most cases only be obtained by enzymatic determination of amylolytic activities in eluates from polyacrylamide gel (see below) Only in a few samples were those 2 isoenzymes present in sufficient amounts to be revealed by activity staining Accordingly, while isoenzymes 1+2 showed as a sharp zone after protein staining isoenzymes 3+4, in the majority of cases, did not

Our experimental data present evidence of amylase congestion in the submaxillary glands of rats during the first week of guanoxane application The concentration of amylase in the glands was distinctly higher in treated than in untreated animals We observed the same effect in parotid glands of guanoxane-medicated rats (Arglebe & Chilla, 1975) Amyloclastic activities were enhanced both in gland extracts (total amylase concentration Table I) and the 4 α amylase isoenzymes (Table II, Fig 2) With the exception of isoamylases 3+4 on the first and third day, the increment rates are statistically significant Protein concentrations, however, do not change during the first week, nor do they rise

Table II *Isoamylases Relative protein amounts total and specific enzyme activities*

Values for total activity are based on the average diagrams of Fig 2 For specific activity see explanation in Table I S C U =Street–Close units

Group	Days treated	Days untreated	Isoamylases 1+2 (0–18 mm) Total activity[c] (ΣS C U ×10³)	Isoamylases 1+2 (0–18 mm) Relative protein amount (mg peak area)	Isoamylases 1+2 (0–18 mm) Specific activity[c] (×10³)	Isoamylases 3+4 (18–36 mm) Total activity[c] (ΣS C U ×10³)
I (n=7)	1	–	10 0 (5%[b])	108	123	25 7
II (n–7)	3		25 0 (10%)	145	237	23 3
III (n 7)	7	–	41 0 (2%)	131	382 (1%)	56 8 (2%)
IV (n–5[a])	21	–	21 0	155	161	30 0
V (n–6[a])	7	21	13 0	143	110	9 0 (2%)
VI/VII (n=10)	–	7/21	17 0	151	132	14 8

[a] 2 rats (group IV) resp 1 rat (group V) died after injection
[b] Difference to control Levels of significance ($p \leq n$ %)
[c] For the sake of perspicuity values were multiplied by 100

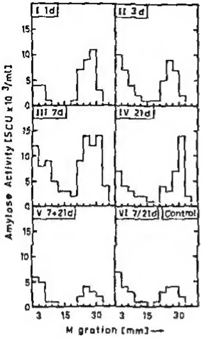

Fig 2 Distribution patterns of isoamylase activities after electrophoresis of each 2 μg protein from submaxillary gland extracts (average diagrams) These diagrams were computed by averaging all single diagrams obtained for each group Street–Close units (S C U) determined in each 3 mm gel section were multiplied by 1 000

above control values This applies not only to extracts but also to electrophoretically separated samples It is interesting to note that rotein contents are about twice as high as se measured in parotid gland extracts Pro- n' values remaining unaltered, the specific mylase activities of extracts and separated isoenzymes, too, increase during the first week, and, for the most part, statistically significant (Tables I and II) However, specific isoamylase activities could only be determined for isoamylases 1+2 as protein staining, mandatory for the calculation of specific activities, could not be achieved for isoenzymes 3+4 Following electrophoresis, merely 9 out of altogether 42 gland samples showed at least traces of bands in the gel region where those two isoenzymes would be found In most cases, charging the gels with even more than 100 μg protein failed to reveal an appropriate zone after staining with Coumassie Blue The amylase assay of Street & Close (1956), however, always detected distinct amylolytic activities for all 4 isoamylases Therefore at least total amylase activities could be meas ured for isoenzymes 3+4 (Table II and "aver age diagrams" in Fig 2) It must be under stood, however, that the specific amylas activities of the gland extracts (based on *rea* protein concentrations) cannot be directl compared with the specific isoamylas activities which are based on *relative* protei amounts

Amylase congestion as demonstrated by th increased enzymatic activities has disappeare after another 2 weeks of continued treatmen with guanoxane (group IV), and protein ha even decreased slightly, but significantl (Table I) When drug application for one weel had been followed by a 3 week recovery phas (group V), all three parameters measured were below the control levels

Since the control groups did not differ significantly, they were combined into one group of 10 animals

DISCUSSION

Guanoxane blocks the transmission of im pulses in the postganglionic sympathetic nerve fibres (Peart & MacMahon, 1964, Davey & Reinert, 1965) Today we know that the secretion of α amylase from the acinic cell is induced by stimulation of β adrenergic re ceptors (Schramm et al , 1965, Babad et al , 1967, Batzri & Selinger, 1973, Schramm & Selinger, 1974) Therefore, elimination of the sympathetic innervation of the gland should be expected to cause congestion of amylase in the secretory granules Amylase synthesis itself is not affected by such treatment because it is operated mainly through parasympathetic innervation (Schneyer & Hall, 1966, Wilborn & Schneyer, 1972) Our findings in the sub maxillary glands during the first week of treatment confirmed this theoretical assumption As in the rat parotid gland (Arglebe & Chilla, 1975), the proportion of α amylase of total protein extracted from the glands

ncreases drastically under guanoxane medica an The increment rates are statistically y significant These changes go parallel th an increased activity of all 4 isoamylases which reaches the maximum after 1 week

Three weeks after the rats had first been in ected with guanoxane, increased amylase activities were no longer found in the submax- y glands Similar observations were re by Schneyer & Hall (1966) after 'sur- al' and by Arglebe & Chilla (1975) follow ' pharmacological sympathectomy of the at parotid glands We assume that also after ympathetic denervation of the submaxillary an amylase secretion develops which is dependent of sympathetic innervation and hich is triggered by the still intact receptors of the acinic cells (Arglebe et al , omitted for publication) In reacting hyper- sitively to minute amounts of the neuro transmitter the denervated salivary lands closely resemble other denervated tis es such as muscle (Gutmann 1962, Emmelin Trendelenburg 1972) With β receptors locked such secretory automatisms of mylase which are independent of sympathetic iervation cannot develop (Arglebe et al ubmitted for publication) As the corre ding parotid gland the guanoxane treated axillary gland of the rat can be regarded a model of the peripheral type of a neuro- sialosis

There is one aspect in which the rat sub axillary gland differs from the parotid gland following guanoxane application The amylase of the parotid gland increases as red with the controls after a 3 week ation phase following treatment for 1 week We explained this phenomenon as a reaction of the gland to the recovery phase Under the same conditions the enzyme content of the submaxillary glands is lower than that of the controls The drop of activity is caused by disappearance of isoamylases 3 and 4 We obtained similar results for submaxillary glands of rats following clonidine application (Chilla et al 1975) This drug also decreased the activity of isoamylases 3 and 4 when treatment had been followed by a recovery phase The activity of the same isoenzymes decreases in senescent rat parotid glands (Chilla et al , 1974) In both instances the amounts of isoamylase protein do not change which supports the assumption that either the gland synthesizes less active amylase or that the enzyme loses activity after its synthesis Isoamylases 3 and 4 of the submaxillary gland seem to be very sensitive in this respect Although their activity could be measured after electrophoretic separation, it was impossible to demonstrate protein by staining because its concentration was too low even though the relatively large amount of 100 µg protein had been applied onto the gel Thus, only total isoamylase activity but not the corresponding specific enzyme activities could be evaluated statistically Further studies might indicate whether *structural peculiarities* are responsible for the described loss of activity Clinical studies on alterations of isoamylase patterns in human saliva can possibly be employed for a diagnostic characterization of different types of sialoses In any case, changes in concentration and distribution patterns of isoamylases can be demonstrated in the biochemical models of peripheral and central types of sialoses

ZUSAMMENFASSUNG

Ratten wurden mit dem Guanethidinderivat Guanoxan (Envacar®) behandelt und in den aus den Gll submandibulares gewonnenen Extrakten die Aktivität der α Amylase sowie die Proteinkonzentration bestimmt Nach Auftrennung der Drüsenextrakte durch Elektrophorese in Polyacrylamidgel wurden die Isoenzymmuster der α Amylase in gleicher Weise [illegible]

[illegible] Amylaseakt [illegible] spezifische enzymatische Ak [illegible]

Diskussion gestellt Veranderungen im Verteilungsmuster der 4 α Isoamylasen zur klinischen Charakterisierung verschiedener Typen neurogener Sialosen heranzuziehen

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